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PROCEDURES FOR SAMPLING DOLPHINS: A HANDBOOK FOR SHIPBOARD OBSERVERS

Albert C. Myrick, Jr.

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U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Southwest Fisheries Center

NOAA Technical Memorandum NMFS

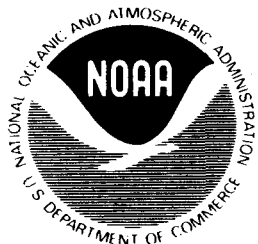
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NOAA Technical Memorandum NMFS

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INTRODUCTION

Every year, since the late 1950's when purse-seine fishing on dolphins to catch yellowfin tuna was first introduced, there has been an incidental kill of dolphins by purse seiners in the eastern tropical Pacific Ocean (ETP). As part of an effort to evaluate the biological consequences of this inadvertent "take" on the affected dolphin populations, biological technicians (observers) of the National Marine Fisheries Service (NMFS) and the Inter-American Tropical Tuna Commission (IATTC) accompany the seiner fishermen on selected cruises to collect biological samples and data from dolphins that are killed. Upon completion of each cruise, the observer delivers his samples to the NMFS laboratory of the Southwest Fisheries Center (SWFC) in La Jolla, California, for study. These samples and data are important to the scientific monitoring effort because they represent all of the life history data that NMFS scientists have to work with in assessing the status of these wild dolphin populations.

Because the life history samples are studied together, it is vital that all samples be comparable. This requires that they be collected with as much uniformity as possible by all observers. Divergent methodologies increase the risk of sampling error. For example, if each observer chose his own method or equipment for measuring body length, a considerable variance in the data could result. Square measurements of a straight body axis might provide a true approximation of maximum length, while tape measurements following the curvature of the body would produce highly erroneous values. Conceivably, length measurements of the same adult taken by different methods could differ by many centimeters depending on the method and the equipment used. Because one of the criteria for distinguishing between certain ETP dolphin stocks is average adult length, the substantial error introduced by various methodologies in collecting length data could render the length criterion useless for stock discrimination. Thus, for body length measurements and for many other aspects of data collecting and specimen sampling, standards are needed to minimize procedural drift by observers.

This handbook was written to provide procedural guidelines for shipboard observers in collecting dolphin organ and tissue samples and associated data. Its focus is on current and potential sampling needs of the SWFC and IATTC. The handbook contains a description of the sampling equipment and its proper use and care. It covers a number of sampling situations and explains the priorities that must be considered in deciding what sort of collection to make. Elements of some organ systems are described and illustrated in this handbook, but it is not intended as a complete atlas of dolphin anatomy. (I have appended a short reading list for those readers interested in more thorough treatments of mammalian and cetacean anatomy.) Finally, included in the handbook is a suggested collecting routine that minimizes specimen processing time without sacrificing sample integrity. Although the handbook was prepared with the observer trainee in mind, others should find parts of it useful also. It is indexed for ready referral to key-word topics.

SAMPLING EQUIPMENT AND USE

The sampling equipment described in this handbook is the equipment that is regularly issued to observers who accompany tuna purse seiners.

Porpoise Life History Form

A Porpoise Life History Form (the form) is used to report biological and other field data for each dolphin carcass processed (Fig. 1). The form is your most important sampling tool. Use it to remind yourself of what you have and have not done in sampling a specimen.

The importance of the data contained on a completed form cannot be overemphasized. Used in concert with other reporting documents, such as the Marine Mammal Set Log and the Marine Mammal Sighting Record, the form provides researchers with information not only about the stock, sex, body length, and reproductive condition of the sampled specimen, but also about the other dolphins associated with it, and where, when, and under what conditions they were together. When finally completed at the lab, the form virtually **becomes** the biological specimen that the researchers analyze. For that reason, it is imperative that all observers complete the form in the same way, with a strict understanding of what each item calls for and how to respond to its requirements.

1. Guidelines for Completing the Form

The form consists of two sections: the in-field section (the upper half) and the in-lab section (lower half). You should complete the in-field half of the form with a **soft-lead pencil** to record the field data for each specimen processed. Each item of the in-field section is followed by a line with non-coded and

[illegible]

Figure 1. Porpoise Life History Form

coded divisions (see Fig. 1). Only non-coded responses should be made in the field. All coding and completion of the in-lab section of the form is done by technicians at the La Jolla Laboratory. To ensure that other persons will have no difficulty in reading your records, you should print all data and all names and words (except compass directions and dates) in unabbreviated form. For example, print and spell out "Stenella attenuata" instead of using "S. attenuata"; spell out "female" rather than using "f" or the biological symbol for female.

If you make an entry error, **do not erase**; line through the error with a single line and add the correct data beside or above it. Records of errors are often useful to data editors and researchers in rectifying cases of mixed-up specimens or data.

You should respond to all items in the in-field section of the form appropriate to the specimen that you are processing. This means, for instance, that if the specimen is a male, responses are required for all non-sex dependent items, such as "SPECIES/STOCK," "HEAD," and "TOTAL LENGTH (cm.)," and for the male-dependent item "TESTIS," but female-dependent items, such as "OVARIES & UTERUS," "LACTATING ?," or "FETUS," should be left blank.

The **only** situation in which you would not respond to all appropriate items on the form is when you collect the whole carcass. If you collect the carcass because there are more carcasses than you can measure and physically sample within the time limits set by the skipper, fill in only lines one, three, and four of the form, plus the item "CARCASS." If, however, you collect a carcass because of some special request or standing requirement and you have the time, you should measure total length, take a jaw sample containing teeth, and fill in the top five lines of the form, plus the items "TEETH" and "CARCASS." (The tooth sample is taken in the latter case to ensure that the specimen will be represented by at least a tooth sample if the carcass becomes lost after the contents of the ship's wells are unloaded in port.)

2. Explanation of the Data Items: Definitions and Responses

The in-field items are explained below in the order that they appear on the form. Item titles are listed exactly as they appear on the form, from left to right, top to bottom, except for my notes enclosed in brackets (see Fig. 1).

CR.# [cruise number]

This item asks for the unique cruise number that identifies your cruise. A cruise number is assigned sequentially to each observer-accompanied cruise by the NMFS San Diego Field Office. The cruise number is usually transmitted to the observer by radio shortly after the seiner leaves home port because of the uncertainty of departure times and the need to preserve the chronology of cruise numbers.

SPECIMEN # [specimen number]

The specimen number consists of a unique three-letter group, followed by a four-digit number. The three-letter group is assigned to you permanently by NMFS when your observer training is completed. You will assign the four-digit number to a specimen, but the number is **not a valid specimen number without your three-letter prefix**. If you are assigned letters ABC and on your first cruise you process your first carcass, you will assign it specimen number ABC0001. If you process a second specimen on your first cruise, you will assign it specimen number ABC0002, and so on. On your next cruise in which there is mortality, the first specimen that you process will receive ABC and the next four-digit number following the number that you assigned last on your first cruise. In this way every processed specimen is represented by a unique field number, and because letter groups are different for each observer, no two specimens should have the same specimen number.

You should assign a specimen number to a specimen after you have processed it and have filled in at least the first five lines of the form for the specimen. You should also assign a specimen number to a carcass that you have collected whole and tail-tagged, i.e. secured a cable tie bearing specimen identification to the base of the tail (tail-stock) just in front of the flukes. In this case, you must also copy onto the upper left corner of the form any other identification number on the carcass's tail-tag.

SPECIES/STOCK

For this item, you should print the full scientific name (genus and trivial) and stock name according to the established field criteria. If you are uncertain about the stock identification, the scientific name should be followed by the word "unidentified" and you should try to take photographs of the specimen. Because species and stock identifications are based primarily on observations of the school composition, your entry for this item should be one of the names that you have provided on other reporting documents for the same set on the school.

SEX

Print out in full either "male" or "female" in response to the implicit question: "Which sex is this specimen?" Be sure that you understand the procedure for determining the sex of a specimen. It is described below under "EXTERNAL ANATOMY ITEMS."

DATE (YR/MO/DAY)

This item refers to the year, month, and day in which the specimen was killed. You should indicate the year with four digits, the abbreviated name of the month, and the day of the month indicated with two digits. For example, for a specimen killed on 2 January 1987, your entry should read: "1987/Jan/02."

SET # [set number]

Each time the net is "let go," i.e. the skiff with net connected is launched from the stern of the seiner, a set is considered to have occurred. On every cruise, each set, regardless of whether it is a "marine mammal set," is numbered sequentially beginning with the number 001. If, for instance, your first set was a school-fish set, your second set was a marine mammal set that involved no mortality, and your third set resulted in 12 specimens for processing, the form for each specimen processed should have "003" entered for this item.

POSITION (LAT/LONG)

For this item, furnish the coordinates, in degrees and minutes, of the set in which the specimen being processed was taken. For example, if the coordinates of the marine mammal set were 14 degrees 30 minutes north, 120 degrees 15 minutes west, you would enter "14° 30'N/120° 15'W" next to this item.

QUAD [quadrant]

This item asks you for the quadrant of the hemisphere in which the set was made. For the eastern tropical Pacific, the correct response for sets made above the equator would be "N/W," and for sets below the equator it would be "S/W."

TOTAL LENGTH (cm.)

Basic specimen data include a total body length of the carcass, rounded to the nearest whole centimeter. For example, if the specimen were 138.4 cm long, you would enter "138;" if it were 159.5 cm long, you would enter "160." See "Measuring Devices" for instructions on making the body length measurement.

(SPOTTED ONLY)
COLORATION

Among the dolphin stocks involved in the yellowfin tuna fishery, only spotted dolphins undergo several distinct changes in pigmentation (color phases) as they mature. The stages are classified as: 1. neonate (dark cape, white ventrally and laterally, with total body length under 100 cm), 2. two-tone (similar to neonate, but over 100 cm and grayer ventrally than the neonate), 3. speckled (spotted ventrally and laterally), 4. mottled (ventral and lateral spots touching and merging, dorsal spotting), and 5. fused (ventral and lateral fields gray monotone, spots dorsally). Use one of these classifications in the blank. Complete this item only if you are processing a spotted dolphin, Stenella attenuata. Leave this item blank for any other species.

LACTATING?

This is a sex-dependent item and you should complete this blank with a "yes" or a "no" **only** if the sex of the specimen is female. The item asks: "When you checked the mammarys, was the female lactating?" The two-step test for lactating is described below under EXTERNAL ANATOMY ITEMS. To leave this item blank when the specimen is a female means that you did not check for lactating. (So, don't unless you didn't!)

FETUS: SEX

Complete this blank by printing the sex of the pregnant female's fetus **only** if the fetus is 25 cm or more in length and you discard it. In other cases, no response is required. Fetuses less than 25 cm long are not sexed or measured in the field, but are collected with the reproductive tract of the pregnant female (see note at bottom of in-field section of form, Fig. 1).

[FETUS] LENGTH (cm.)

In this blank, you should enter the total body length measurement of the pregnant female's fetus, to the nearest whole centimeter, **only** if the fetus is 25 cm or more in length and you discard it. In other cases, no response is required.

COLLECTED?: TEETH

You should collect eight to ten teeth from the middle of the tooth row of the left mandible (i.e. the left lower jaw). Answer "yes" if you collect the teeth; otherwise answer "no." A jaw sample containing teeth should be collected separately when the head is collected or when a carcass is collected because of special or standing instructions.

[collected?:] TESTIS

A response to this item is required only if the specimen that you are processing is male and you do not collect the carcass. If the specimen is male, the proper response is "yes" if you collect the right testis or "no" if you do not.

[collected?:] OVARIES & UTERUS

This item requires a "yes" or "no" response only if the sex of the specimen is female and the carcass is not collected. A "yes" response indicates that you collected the entire reproductive tract.

[collected?:] FETUS

This item asks: "Did you collect a fetus?" You should respond only if the adult specimen is female and after you inspect the uterus from the outside to try to ascertain the presence or absence of a fetus. A "yes" to this item indicates that you observed (through the uterine wall) or felt what you interpreted to be a fetus, that it was less than 25 cm long, and that you collected it along with the reproductive tract. A "no" response indicates that you did not collect a fetus although it does not necessarily mean that no fetus was present. (Current requirements instruct that all fetuses 25 cm or more in length should be sexed, measured, and discarded.) A third response to this question may be a question mark. A question mark indicates that you checked, but that you were not able to confirm the presence or absence of a fetus. **Caution:** Do not open the uterus to look for a fetus unless you are certain that it is 25 cm or more in length. A small embryo may be lost through the opening that you make. An unpunctured uterus ensures that its contents are collected intact.

[collected?:] STOMACH

This item requires a "yes" or "no" answer to the question: "Did you collect the stomach from this carcass?" Do not leave this item blank unless you collect the carcass.

[collected?:] HEAD

If the carcass is not collected, this item requires a "yes" or "no" answer to the question: "Did you collect the head?" Leave the item blank **only** if you collect the carcass. If you collect the head, you should collect a jaw sample separately.

[collected?:] CARCASS

If you collect the carcass, you should answer this item with a "yes;" otherwise answer with a "no." Do not leave this item blank. If you collect the carcass for purposes other than to save time, you should collect a jaw sample separately.

[collected?:] MAMMARY GLAND

If the specimen is female, this item should have either a "yes" or a "no" response depending on whether you collect a tissue sample from the mammaries. No response is required if the specimen is male, or if you collect the carcass.

[collected?:] MILK

If the specimen is not a lactating female, or if you collect the carcass, you need not respond to this item. Otherwise, a "yes" or "no" answer to the question: "Did you take a milk sample from the mammaries?" is necessary.

[collected?:] PARASITES

This item should not be left blank unless you collect the carcass. You should provide a "yes" or "no" answer to the question: "Did you collect any parasite from any part of the carcass?"

[collected?:] BLOOD

This item asks: "Did you take a blood sample?" You should respond with either a "yes" or a "no." Do not leave this item blank unless the carcass is collected and blood is not collected from it separately.

PHOTOS? ROLL # FRAME(S)

These three items are concerned with the question: "Did you take photographs of this specimen and, if so, how many exposures were taken and what was the number used to identify the roll of film?" The item "PHOTOS" must be answered with a "yes" or a "no." If your response to PHOTOS is "no," do not respond to the following two items. If it is "yes," then you should furnish the roll identification and the numbers of frames exposed. No response to these items should be made if you collect the carcass.

COLOR PATTERN [sketch] & DORSAL FIN [trace]:

To decrease processing time per specimen and maximize sample volume, sketches are not required for spotted dolphins and only the outline of the ventral hump, dorsal fin, and color pattern is required for spinner dolphins. If you are working up a spinner dolphin, your instructions may also require you to trace the dorsal fin on the back of the form (see EXTERNAL ANATOMY ITEMS for procedure).

If you have plenty of time, you may use the sketch outline to illustrate features of interest, such as scarring, unusual pigmentation patterns, or points of injury. Although not a required part of processing, the use of the sketch in this way frequently has proven useful to researchers in the past. If you collect the carcass, do not respond to this item.

BASIS FOR STOCK ID:

This item asks you for the criteria that you used to determine the stock to which the carcass being processed belongs. The criteria used should emphasize the general appearance of the adults in the school from which the carcass came. In cases where it is obvious that a school consists of animals of a single stock, identification is usually straightforward. However, in cases where it appears that more than one form of the same species is represented, you should include in your evaluations such criteria as homogeneity or heterogeneity of behavior between groups in the encircled school.

In any case, the carcass that you are processing should belong to one of the stocks that you identified from observations of the school. You should make clear whether you have identified the carcass strictly according to your observations of the school, without considering the characteristics exhibited by the carcass. If you collect the carcass and do not identify the specimen, leave this space blank.

Measuring Devices

1. Sticks

Two, two-meter measuring sticks and one pair of moveable caliper jaws (Fig. 2) may be included in your equipment package for measuring total body lengths of carcasses and for providing scale in photographs of single carcasses.

Care

The sticks are constructed of wood and vulnerable to breakage and warpage. You should use special care in handling and

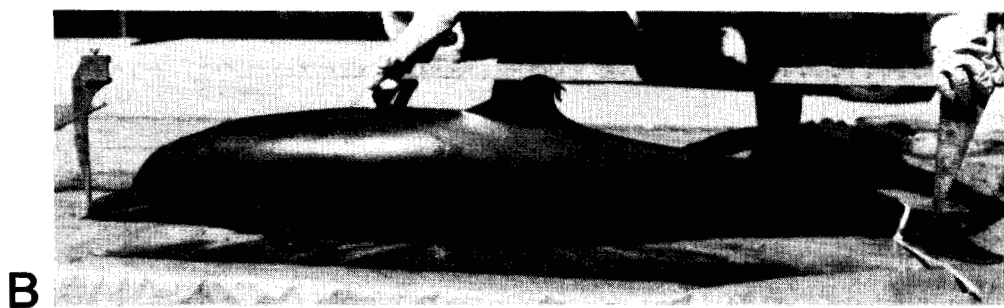
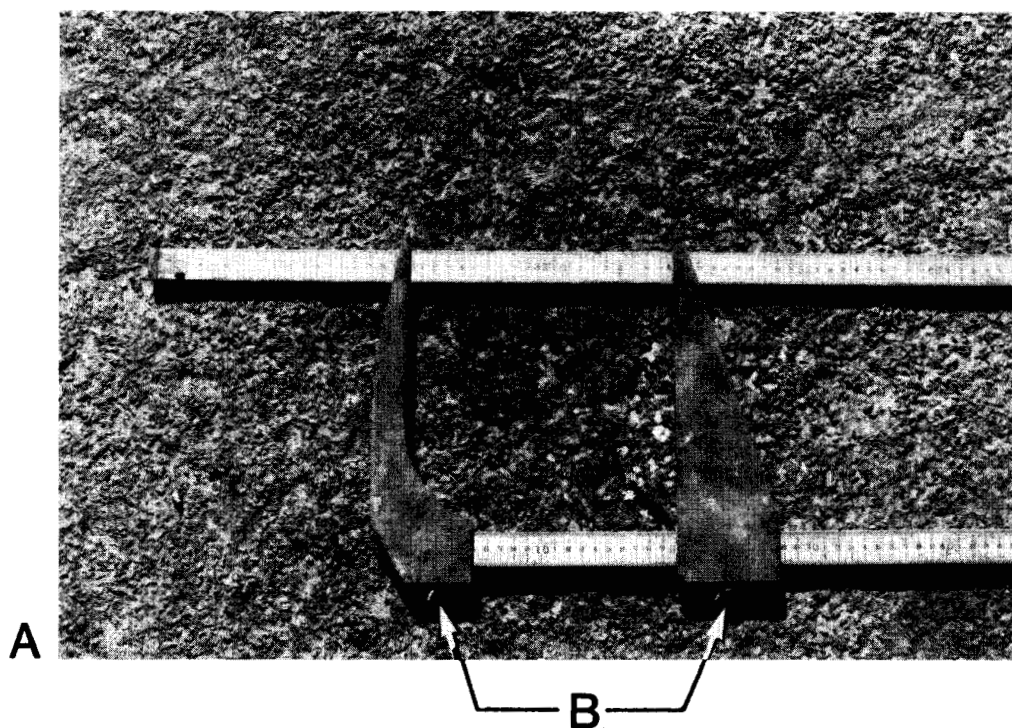


Figure 2. A. Measuring sticks and moveable caliper jaws positioned for calibrating calipers. Arrow at A indicates where the measurement should be read. Arrows from B indicate position of setscrews for locking or adjusting the jaws. B. Taking a dolphin's body length measurement with a measuring stick. One caliper jaw is in the tail notch and the other one is at the tip of the upper jaw.

storing them. To minimize warpage, keep them dry and lubricated with silicone spray (included in your equipment package). And, whether at the workup site or the storage area, lay (or store) them flat when they are not in use.

Metric Measurement

The sticks are marked in inches on one side and in centimeters on the reverse side. Measurements must be made in centimeters, so it is important to cultivate the habit of consciously checking which side of the stick you are reading. On the metric side of most sticks, the numbers go up to 100 cm and begin over again at one. Thus, the number at 110 cm is only "10." Failure to note that the first 100 centimeters has been traversed during carcass measurements could lead to records of remarkably short-bodied animals.

Calibrating the Caliper Jaws Between Sets

Both caliper jaws are moveable and the one nearest the zero end of the stick that remains stationary during measurements, (the "stationary" jaw) may come out of calibration because of a loose setscrew, a sudden jolt in transit between decks, or during storage, simply through vibrations from the boat. To ensure that measurements with the stick are accurate, you should recalibrate the stationary jaw **at the workup site** before processing specimens from a set.

Begin calibration by placing the stick without jaws (the first stick) on the deck, metric side up. Place the stick to be calibrated (the second stick) parallel to the first stick so that the zero ends of both sticks are in the same direction and both caliper jaws of the second stick extend at a right angle across the metric scale of the first stick (Fig. 2A). While keeping the two sticks parallel, slide the non-stationary caliper jaw (i.e. the jaw farthest from the zero end of the stick) to the 20-cm mark of the first stick and to the 10-cm mark of the second stick. The first stick should now extend 10 cm in the zero-end direction beyond the second stick. Tighten the setscrew of the non-stationary jaw. Now, move the stationary jaw to the 10-cm mark of the first stick and tighten its setscrew. Calibration of the second stick should be complete and the stick ready for use.

As a test, loosen the setscrew on the non-stationary jaw, slide the jaw along the stick to its 35-cm mark, and tighten the setscrew. Place the two jaws across the metric scale of the first stick. If the stationary jaw of the second stick is calibrated correctly, the distance showing between the two jaws on the first stick should be 35 cm.

Taking Body-length Measurements

The total body length measurement may be taken with the carcass resting slightly on its right side on the deck. The long axis of the body should be straight. The lower jaw usually extends farther forward than the tip of the upper jaw, but despite this common "underbite," standard total body length measurement is made from the tip of the **upper jaw** to the caudal notch of the flukes at midline.

To measure the carcass with the stick, place the stick above the midline of the long axis of the body, parallel to the deck, with the zero-end pointing posteriorly. Loosen the setscrew of the non-stationary jaw, place the stationary jaw into the notch between the tail flukes, and slide the non-stationary jaw along the stick to the anterior tip of the upper jaw of the specimen (Fig. 2B). After checking to make sure that the stick is parallel to the deck and that the caliper jaws are not closed toward each other so tightly that the body is being bent, tighten the setscrew of the non-stationary jaw and remove the stick from the carcass. Read the measurement from the metric side of the stick, round off to the nearest whole centimeter, and record the number next to TOTAL LENGTH (cm.) on the form.

When you are measuring a carcass close to or more than two meters in length, which is not uncommon, begin by following the same procedure as described above. The non-stationary jaw will not reach to the tip of the upper jaw, so move it to a prominent landmark, such as the anterior margin of the melon. Be sure that the measurement is as square as possible; then tighten the setscrew, turn the stick to the metric side, take the reading without rounding off, and write the number down. Now, move the stationary caliper jaw to the point where your first measurement ended and slide the non-stationary jaw out to a point even with the tip of the upper jaw of the specimen. Tighten the setscrew, take the reading without rounding off, and add the first reading to the second. Now, round off to the nearest whole centimeter and enter this number on the TOTAL LENGTH (cm.) blank.

2. Other Devices

You may be issued equipment other than measuring sticks with which to take length measurements. Flexible tapes with various accessories that permit square measurements have been used. They have several advantages over sticks: 1) they are compact and easily transported, 2) they are less vulnerable to damage, and 3) they do not require recalibrating between sets. Their main disadvantage is that they are flexible and may consistently sag a bit during measuring. This requires the measurer to take special care that the tape is taut and parallel to the deck. Because of the problem of tape sag, it is best to use a rigid measuring stick whenever possible.

Labels, Cable Ties, and Labeling

Among your supplies will be several hundred labels (tags), approximately 6 x 12 cm, with metal eyelets for accommodating ties which are used to attach the labels to samples. The labels are specially constructed to resist tearing, deterioration, and dissolution. Each may already have a string in the eyelet, but because string adds knot-tying to the problem of attaching a label, rib-lock cable ties, also found in your equipment package, are now used. If you remove all of the strings before you reach the fishing grounds, you will have saved time by not having to unstring labels during specimen workups.

Cable ties will be in two lengths: ~ 15 and 60 cm, the shorter for attaching labels to organ and tissue samples, the longer (pre-embossed in packs of 25 or 50) for tail-tagging carcasses. To attach a cable-tie label to a specimen, thread the tie through the eyelet of the label, encircle the organ sample with the tie, then pass the end of the tie through the tie sleeve and cinch it down tightly on the sample to lock it. To ensure that the correct side of the tie is against the sleeve, give a few tugs on the tie to try to back it out. If it backs out, thread the tie back through the sleeve using the opposite side of the tie against the sleeve to lock it down. Test again for locking.

Print the specimen number on the label in **soft-lead pencil**. No other data should appear on the label and no writing implement other than a soft-lead pencil should be used. Finally, all of the labels needed for collecting samples from a specimen may be made up at the same time, **but never make up labels for more than one specimen at a time**. Prenumbering labels greatly increases the chance of data mix-ups.

Gloves

Your equipment includes one pair of heavy cotton gloves, several pairs of Playtex-type rubber gloves, and up to a half-dozen pairs of surgeons disposable latex gloves (optional). **Always wear gloves** to protect your hands from minor injuries while you are handling carcasses and to prevent contact with potentially harmful microorganisms or fixative. The cotton gloves are intended for use in moving carcasses. The rubber gloves are for use during specimen processing and handling of fixative or fixed samples. If the rubber gloves are used for carrying or dragging carcasses, they will puncture or tear. You will have no protection for your hands during specimen workups if all of the rubber gloves have been damaged through misuse, because cotton gloves are porous and too cumbersome for removing most anatomical samples.

Containers, Preservative, and Specimen Handling

1. Buckets and Bags

You will be taking aboard up to three plastic five-gallon buckets with watertight lids, small and large plastic bags and burlap bags. Buckets are for: 1) receiving samples being collected on the working deck, 2) formalin-fixation of samples, and 3) specimen storage and boat-to-lab transport of consolidated preserved samples. Plastic and burlap bags are for packaging specimens that require frozen storage.

2. Fixative: Preparation and Cautions

A one-gallon plastic bottle containing 100% formalin (a saturated solution of 37% formaldehyde) is part of your regular issue. A 10% formalin solution is needed for optimal fixation of tissues (a process that may take about one week depending on tissue thickness and permeability). Concentrations greater than about 13 or 14 % usually result in formalin "burn" (actually a bleaching/hardening process), so be sure to follow the recipe.

To prepare the fixative, mix one part (100%) formalin to nine parts seawater from the deck hose. (For the sake of maintaining congenial relations with the crew, it is best not to use the boat's limited freshwater supply for making fixative or for cleaning tools or specimens.) Prepare enough 10%-strength solution to fill one bucket about half full. Keep this bucket of fixative in the storage area for receipt and complete fixation of the freshly collected samples. (One of the best places for storage of specimens, data forms, and collecting equipment is near the bow on the wet deck because it is out of the way of heavy traffic, and despite its name, the wet deck is usually dry.)

Avoid skin contact with the fixative or prolonged inhalation of its fumes. Besides being an irritant to skin, eyes, and nasal membranes, formaldehyde is generally considered to be a potential occupational carcinogen that is known to cause certain kinds of cancer in laboratory animals. Remember to wear rubber gloves when preparing fixative, or when transferring fixative or fixed specimens from one container to another. **Handle the fixative only in a well-ventilated area.** If you can smell the formaldehyde, you may be inhaling enough to do you harm.

3. Handling and Preservation of Samples: Recommended Routine

You will be preserving samples in two ways: frozen storage and fixed-wet storage. Carcasses, heads, and stomachs should be frozen after being collected: carcasses and heads, because they are too large for buckets and they cannot be fixed on board, and stomachs, because formalin is acidic and will etch or dissolve fish otoliths and squid statocysts that are contained in the

stomachs. Frozen specimens smaller than heads are normally stored in the cook's food freezer. You must obtain permission from the ship's cook to use freezer space for storage of samples. Once permission is obtained, try to make any samples stored there as presentable as possible. Carcasses and heads are stored in the wells (where the tuna are stored) with the permission of the skipper or chief engineer. The heads are generally tied to the coamings of the wells. To remind yourself where you have stored your frozen samples, keep a running inventory of them in your green book.

When you collect a head, put a label in the mouth and cinch the jaws tightly shut with a cable tie or strong cord (a length of seine-net twine will do). Make a slit through the skin and muscle near the base of the head and pass a cable tie through the slit. Attach a label to the tie and cinch it securely. Place the head in a large plastic bag and tie it shut with twine to prevent leakage of blood. Finally, place the package into a burlap bag to protect it and use a cable tie (with an outside label to make it identifiable) to close the bag.

Before removing the stomach from a carcass, seal off each end of the stomach with a 15-cm cable tie (one with a label) to avoid spilling its contents. After removing the stomach, place it in a small plastic bag and close the bag with a cable tie and outside identifying label.

Jaw samples with teeth and testis or uterus and ovaries samples are preserved in formalin. Each of these samples should be labeled once with a cable-tied label and placed in your sampling bucket without additional packaging.

If a kill set includes carcasses from which stomachs, heads, or other samples needing frozen storage are required, take your sampling equipment, a supply of bags, and an empty bucket (sampling bucket) up to the working deck with you. (If no frozen-stored samples are to be collected, do not take bags.) Fill the sampling bucket half full of seawater from the deck hose and use the sampling bucket to store tooth and gonad samples until specimen processing is completed. To keep your forms clean and to minimize slippage of hand tools during sampling, use seawater from the deck hose to wash your hands in between workups and data recording.

After you have processed the specimens from a set, take the samples packaged for freezing to the freezer or to the well. Then take your sampling bucket down to the specimen storage area and transfer the samples to the fixation bucket. Keep the lid tight on the fixation bucket to prevent evaporation of fixative and leakage of its fumes. You should always keep enough fixative in the bucket to cover all specimens and the specimens should remain for at least a week in the fixative. After fixation is complete, transfer the specimens to the storage bucket to make the fixation bucket available for new samples. The storage bucket should be lined with doubled plastic bags with only a little preservative

to keep specimens moist. Intermittent monitoring and maintenance of the fluid level in the storage bucket will ensure that the samples do not dry out. If the take becomes high and sets with large kills become frequent during a cruise, it may be necessary to add another fixation bucket to your system. You may substitute a large plastic bag for your sampling bucket and use the sampling bucket for fixation.

At the end of the cruise, close up the double bags of consolidated specimens in the storage buckets with cable ties and close the lids tightly. Cable-tie a label onto the handle of each bucket with your **last name** and the **cruise number** printed on it in soft-lead pencil. As an extra precaution against misplacement of the buckets, use one of the waterproof markers, included in your equipment package, to print your last name and the cruise number on the lid and on the side of each storage bucket.

You will find it convenient during a cruise to keep all (**separately packaged**) stomachs together in the freezer in a large plastic or burlap bag. As you collect more stomach samples, just add them to your stomach bag. At the end of the cruise, cable-tie a label to it with your last name and cruise number printed in waterproof marker. Make sure that there are outside labels with the same information for any heads that you have collected.

Pruning Shears

One of your collecting tools will be a pair of pruning shears for use in collecting jaw samples containing teeth. The blades of the shears are subject to corrosion from salt water and the edges tend to dull from shearing bone and connective tissue. Dull and rusted shears slow the jaw sampling procedure and prolong the overall processing time. After processing specimens, clean the blades of organic materials, dry the blades, and spray them with silicon. If they become heavily rusted, ask permission of the chief engineer or others in charge to use the wire wheel on the bench grinder to burnish the blades. (**Use eye protection while using the bench grinder.**) The shears may be sharpened with the whetstone, included in your pack, or with the ship's bench grinder or any flat metal file (be sure to ask permission to use the grinder or a file).

Knives

Two boning knives are your standard-issue dissection tools. The knives should be kept sharp if they are to be used effectively. This means that practice with the whetstone (included) and oil is important. A crew member may sometimes offer to show the beginner the proper way to set a knife edge. Take him up on it! A dull-edged knife can be extremely dangerous because too much uncontrolled pressure must be applied to cut with it. In slicing a soft tomato with a dull paring knife, it is

easier to cut fingers than to cut tomato. In processing specimens, if you apply pressure to a dull-edged knife against a carcass, and the carcass, your hand, or your knife slips, you may be seriously cut. A knife wound may require the services of a physician, which are usually in short supply on tuna seiners.

Of course, even when they are sharp, knives should be used with care. During processing, you should always embed them in the heavy musculature of the carcass when they are not being used. However, do not forget to remove the knives from the "knife holder" before it is discarded or you may run out of knives long before you run out of carcasses. Do not hold the knife while trying to adjust the position of a carcass. A sudden slip may cause an injury. Hold the knife only when you intend to cut with it. When you cut, you should always keep the point and sharp edge of the blade turned away from yourself.

COLLECTION EFFORT AND PROCESSING DECISIONS

Processing or collecting all specimens brought on board after each set should be high on your list of priorities. Based on recent estimated kill rates, one hundred percent of the take must be sampled to obtain an adequate annual sample for monitoring the affected dolphin stocks. To support this effort, NMFS has offered to reimburse U.S. boats at tuna prices for well space to store dolphin carcasses that cannot be processed by the on-board observer.

For sets in which fewer than 15 or so animals are taken, complete workup on all carcasses is usually not a problem as long as there is no impending set. Processing of so few carcasses should require two to three hours of work. With larger kills, however, certain factors such as time of day, fishing success, and weather must be considered in deciding how many specimens you have time to work up and how many you should tag and send to the wells for processing on shore after the cruise. You must decide this while the chute to the wells is still set up. If, after the chute is dismantled, you have retained more carcasses on deck than can be worked up before a new set is initiated, you will have no choice but to dump some overboard to clear the deck. On the other hand, if you hedge your bet by always retaining on deck an exceedingly small number of carcasses to work up and by sending all of the other specimens into the wells unprocessed, the costs of well-space rental for unprocessed specimens could rise prohibitively.

If the large kill set is made near the end of the day, it would be reasonable to expect no additional sets for the day and you can usually be certain about this by observing the crew's non-searching activity or by asking the skipper. In that case, you would retain on deck a maximum number of specimens that you could process (up to 30) and send the others down the chute.

If, after a kill set, the skipper gives you a time limit for clearing away specimens from the deck (for whatever reason), you should try to collect or process as many specimens as possible. If the chute is still assembled, you might opt to tail-tag and send all of the carcasses that you have time for down the chute. But, if the chute is dismantled, you should work up as many specimens as time permits.

PARTIAL-SAMPLE SELECTION

In cases where only part of the specimens brought aboard from a set can be collected or sampled, carcasses should be selected for processing arbitrarily, as they come onto the deck, or as the crew has stacked them for you. The purpose of choosing specimens randomly is to try to minimize any age or sex bias in the sample caused, for example, by selecting only the smallest, or the largest, etc. Analyses of samples at the laboratory will include an attempt to reconstruct the size and age distributions as well as the sex ratios of schools that were set upon. By using hundreds of school samples in their analyses, researchers can obtain an age and size distribution and sex ratio of the sampled population. With this information and other age and reproductive data, predictions can be made about the future growth rate of the population from which the samples came.

If, in collecting specimens representing only part of the kill, you choose mainly larger animals, a bias toward adults would exist in your sample. Because males usually grow larger than females, the bias might also be toward males. Such samples not accompanied by a description of the sampling procedure used by the observer might mislead scientists conducting the analyses into concluding that the schools were made up chiefly of adult males, with few juveniles.

COLLECTING CARCASSES FOR SHORE-BASED PROCESSING

The large (~60 cm) cable ties are used for rapidly tagging carcasses that go into the wells for processing ashore. They are designed primarily for situations in which there are more carcasses than you have time to process. You should put aside the specimens you have time to process and then send the remainder to the wells. The large cable ties come in packs of 25 or 50 and each tie has a pre-embossed letter/number code, numbered sequentially. Before passing the first carcass down the chute, detach the first cable tie from the pack and secure it, without a label, around the tail stock. Tail-tag the next specimen using the next cable tie of the pack, and so on. Then process your on-deck specimens.

After all of the carcasses from a set are either processed or tail-tagged and in the wells, prepare a separate Porpoise Life History Form for every cable-tie number used. Record a cable-tie number in the upper left-hand corner of each form and then enter the set number, the date, position, enter "yes" for CARCASS, and assign and enter a specimen number (three-letter, four-number code). No other data should be entered. The remaining blanks on the form are filled in when the tail-tagged carcass goes through the complete workup at the laboratory. For purposes of good bookkeeping and to help keep track of carcasses from each set, enter the beginning and ending cable-tie numbers used, by set, in your greenbook. Also, to facilitate recovery of carcasses when the wells are unloaded, you should include beside each entry, the number of the well that contains the carcasses from the set.

SPECIMEN PROCESSING ON BOARD

It is a good idea to establish a uniform routine for on-board processing of specimens early in your tour. If all specimens are aligned the same way and all are sampled and repositioned always in the same sequence, fewer errors and omissions will be made, more specimens can be processed, and workup time per specimen can be appreciably shortened.

The order in which the following procedures are described need not be the order of the routine you adopt. Try out several systems and decide what order is most convenient and efficient for you. Then keep to that order. Periodically, you should review the sample needs listed in the "Regional Observer Life History Requirements" chart, as well as the procedures described in this handbook. By keeping your system current, you can ensure that your methodology does not drift from the requirements to the extent that the collection of important data is systematically omitted. After you have processed and discarded 123 females and you suddenly realize that you forgot to determine whether they were lactating, it is impossible to retrieve the information.

I recommend a processing order that progresses through the items on the form by operational blocks:

- 1) non-dolphin items (data that can be answered immediately without a specimen),
- 2) external anatomy items (data that can be collected without opening the body cavities), and
- 3) internal anatomy items (data that must be gathered from the cavities).

This or a similar order should be adopted because it is simpler, smoother, and faster than following the order of items as they appear on the form. It allows you to respond to operationally related requirements all at once, without having to change sampling modes repeatedly.

GETTING READY

When you go out on deck with your equipment to work up specimens after a kill set, line up as many carcasses as you have time to do side-by-side and position them slightly on their right sides, body axes straight, heads pointed to your left as you view their left sides (Fig. 3). You should note that this relative position (sketch position) is similar to the sketch outline on the form (Fig. 1). (Remember that if for some reason you can sample or collect only some of the carcasses, select them arbitrarily.) After aligning your specimens, immediately prepare your equipment for work up of the first specimen in line. This includes calibrating your stick on site, filling your bucket with seawater, and inventorying your equipment.

NON-DOLPHIN ITEMS

Take a sharpened soft-lead pencil and enter all of the "non-dolphin" data on a new form:

1. cruise number
2. date
3. set
4. position
5. quad.

EXTERNAL ANATOMY ITEMS

Then, examine the first specimen. With the carcass in sketch position you are ready to respond to the first half of the external anatomy items block:

6. species/stock
7. basis for stock ID
8. (if spotted dolphin) coloration
9. (if spinner dolphin) color pattern...(sketch)
10. photos?
11. total length

Coloration/Sketch/Fin Trace/Photos

If you have identified the specimen as a spotted dolphin, (Stenella attenuata), you should now respond to the "COLORATION" item by entering the color phase of the specimen. If the specimen is a spinner dolphin (Stenella longirostris), you should indicate, by lines on the dolphin outline, the shapes of the ventral hump, dorsal fin, and basic pigmentation pattern (Fig. 4A).

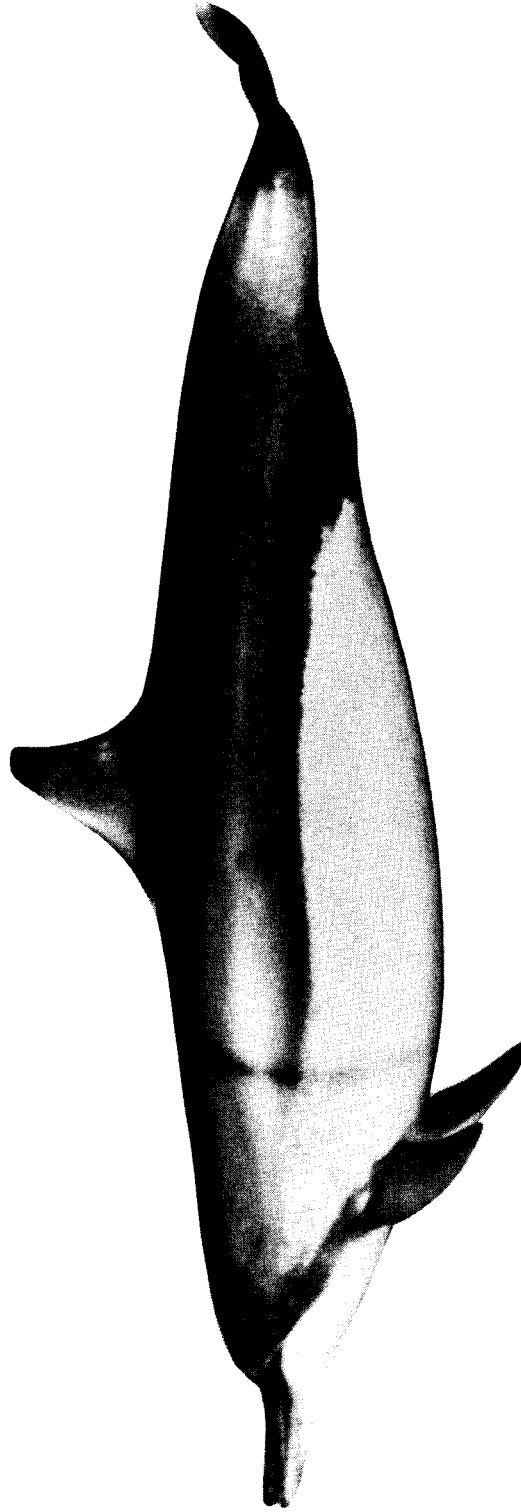


Figure 3. Carcass of dolphin in sketch position (photo taken by J. Greene).

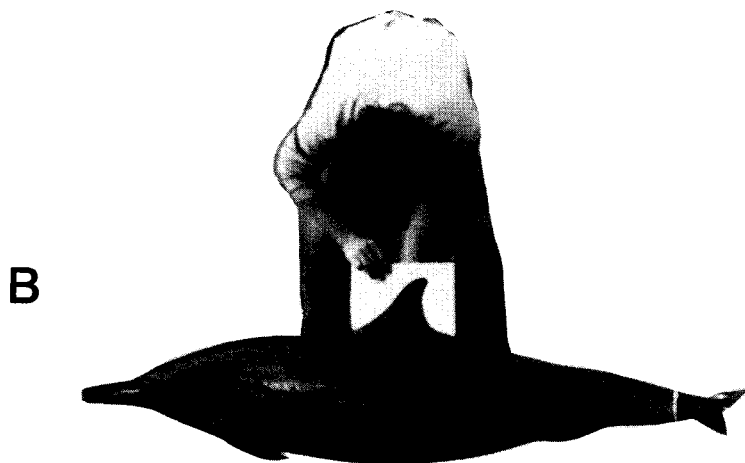
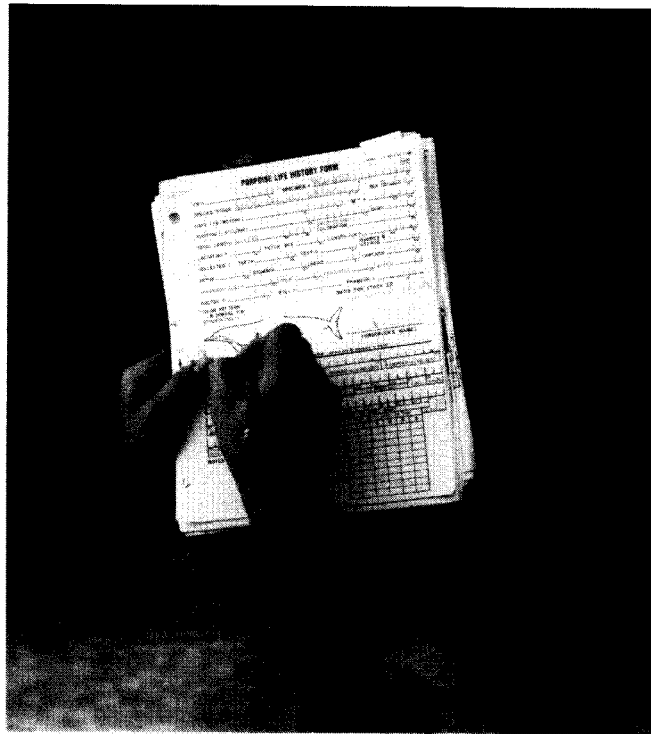


Figure 4. Sketch and fin-trace requirements. A. Outlining the pigmentation pattern on a Porpoise Life History Form to fulfill the sketch requirement for spinner dolphins. B. Using the blank side of the form to trace the fin of a spinner dolphin.

When you are working up a spinner dolphin and requirements include a fin trace, turn the form over to the blank side, place that side behind the dorsal fin, and trace the outline of the fin on the paper with your pencil (Fig. 4B). Include a bit of the margin of the body outline in front and behind the fin so the angle of the fin on the body is evident. If there is not room enough to include the whole fin outline, try to position the paper diagonally. If results are still poor, use backs of two forms placed together (if you do this, make sure that the same specimen number is on both forms). To distinguish anterior from posterior, draw an arrow pointing forward from the anterior margin. Fin traces are used to measure variation in dorsal fin angle among spinner dolphin populations.

If you have some doubt as to the stock to which the specimen belongs, or if the life history requirements so indicate, you should take photographs and enter the photo and film data in the appropriate blanks. Before photographing a specimen, place some sort of scale with it (a measuring stick works well). Finally, be sure that only one specimen is photographed on deck at a time and that the photographing has been approved of in advance by the skipper.

Total Length

Check that the body axis is straight. Now, take the total length measurement. (If you are using a measuring stick, do not forget to read from the metric side and from the inside of the caliper jaw slide (Fig. 2A)). Round off to the nearest whole centimeter and enter the number.

By rolling the carcass fully onto its right side to expose the venter, you are ready to respond to the rest of the block of external anatomy items:

12. sex
13. specimen number
14. (if female) lactating
15. (if lactating) milk
16. (if female) mammary gland
17. teeth
18. head
19. carcass

Sex

Sex

Examine the body along the mid-ventral line, posterior to the level of the dorsal fin. Three criteria should be used together to determine sex from the external anatomy. If the specimen is a female, the genital and anal slits--the two openings on the mid-line of the venter--should be almost continuous (Fig. 5). In males, there is a considerable hiatus between the genital slit and the more posterior anal slit (Fig. 6). A female has a mammary slit on either side of the genital slit, but in males mammary slits seldom develop. When they do occur in males, they are usually found on either side of the mid-ventral line between the genital and anal slits. Finally, to ensure correct sex determination, insert a small blunt-ended probe, such as the eraser end of a pencil, eraser first, into the genital opening of a specimen. Try to push the pencil gently forward, then backward, at a slight upward angle. You should find that if the pencil goes easily forward, you cannot push it backward. If the pencil goes forward it enters the vagina which is angled forward and slightly upward and the specimen is female. In males, the pencil can be moved backward into the chamber that houses the penis, but not forward (Fig. 7).

Specimen Number

Now that you have recorded all of the minimum data necessary for assigning a specimen number, you should print your three-letter prefix followed by the next succeeding four-digit number beside this item on the form. This should be the same as the number on the labels that you are about to make up in advance for the specimen.

Advance Preparation of Labels

Having determined the species and stock of the specimen, you are ready to check the "Regional Observer Porpoise Life History Requirements" to find what samples are to be collected (Fig. 8). Knowing this will permit you to save time by preparing labels (printing specimen number) for all of the samples from the specimen in one operation. But do not number labels for more than one specimen at a time. Prepare labels for the first specimen, work it up completely, then prepare labels for the next specimen, and so on.

For standard workups (in which just the jaw, with teeth, and the gonads are sampled) only two labels are needed. If you collect the stomach, you need two more labels. If the head is collected, you should make up three labels for it. (But remember, if the head is taken, you still must collect a jaw sample separately.) Thus, for a specimen from which the jaw sample, reproductive tract, stomach, and head are collected, you should prepare seven labels (see Table 1).

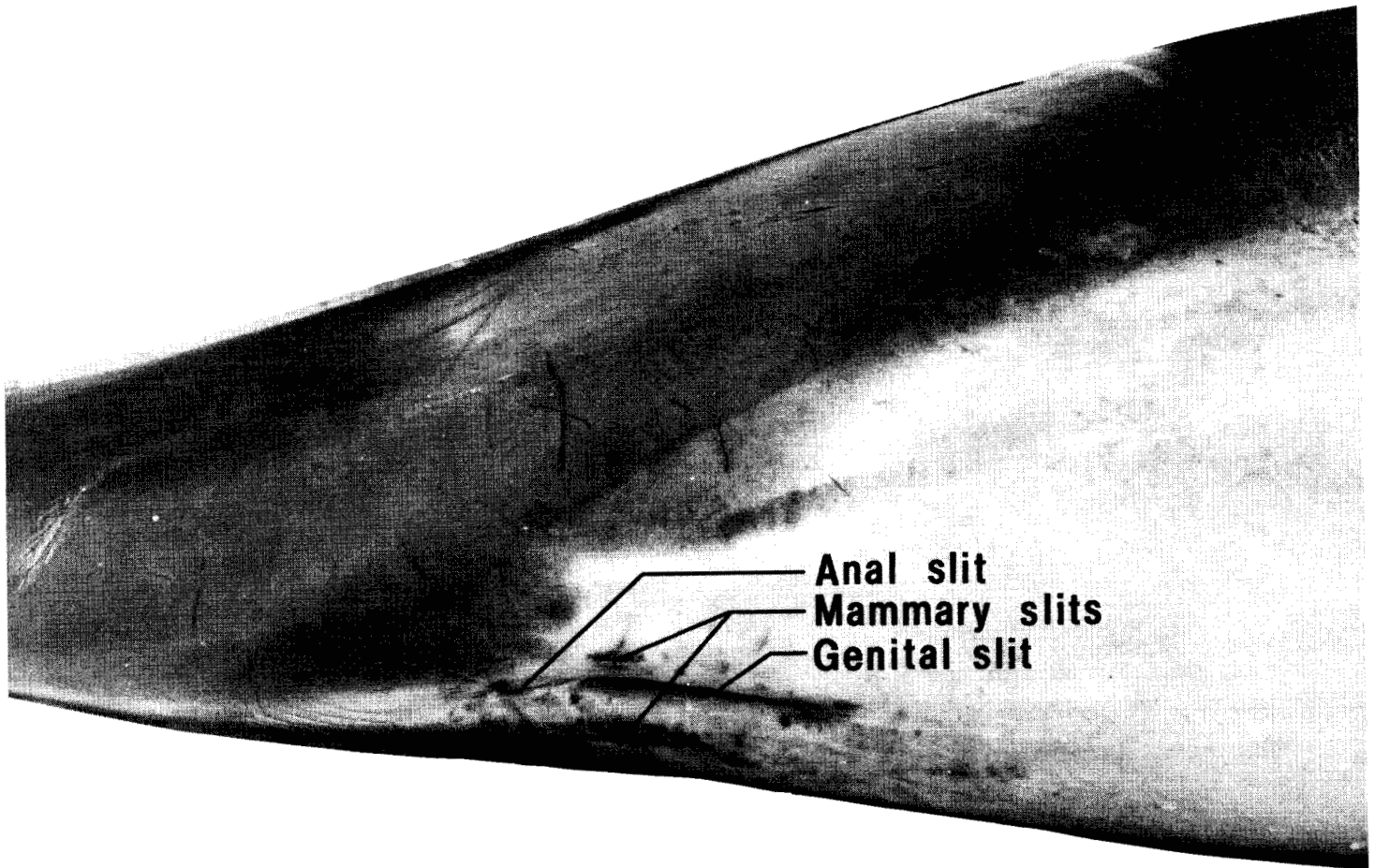


Figure 5. Ventral surface of female dolphin showing genital slit, anal slit, and paired mammary slits. The genital and anal slits are almost continuous in female dolphins (photo courtesy of W. Perrin).

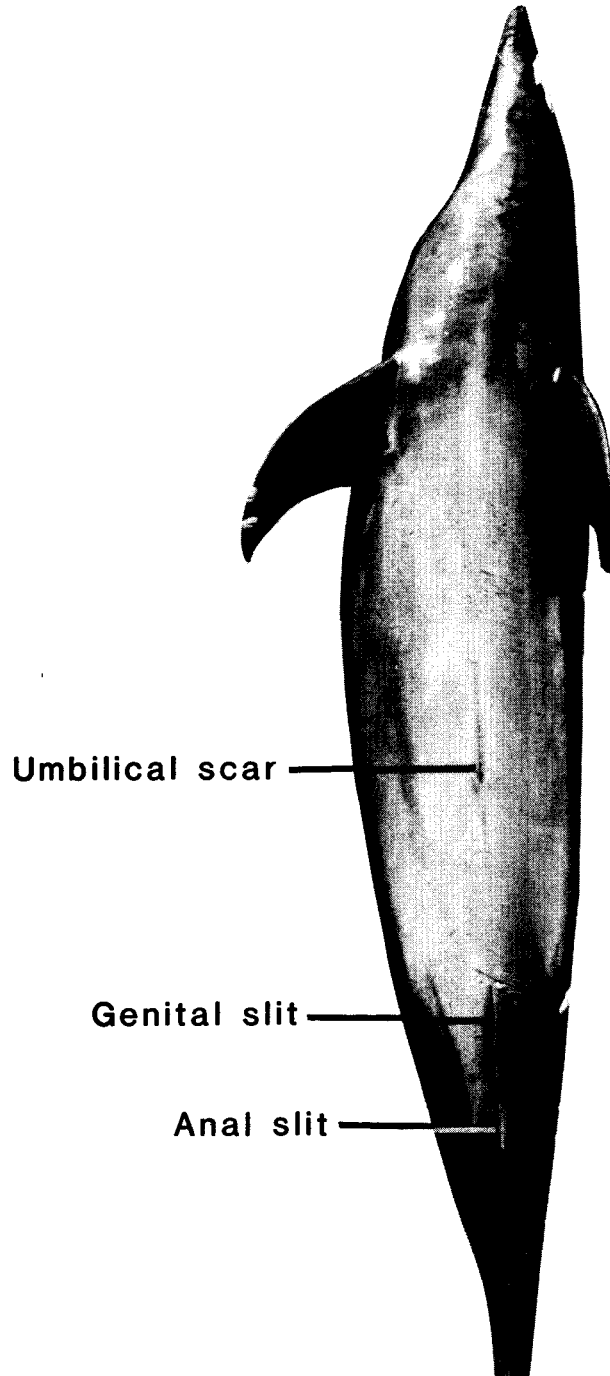


Figure 6. Ventral surface of male dolphin showing the umbilical scar, genital slit, and anal slit. The genital and anal slits are well separated in male dolphins (photo courtesy of W. Perrin).

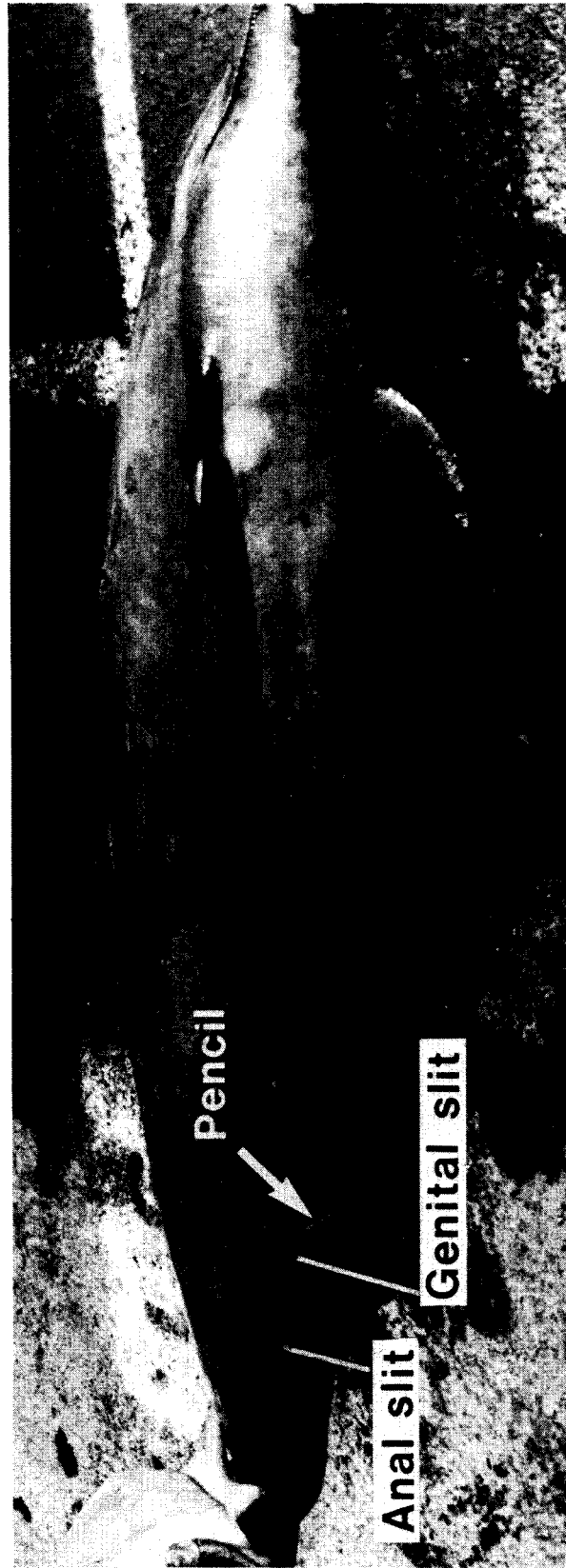


Figure 7. Use of blunt end of pencil to determine the sex of a dolphin. If probe can be pushed backward when inserted into genital opening, the specimen is male. If it can be pushed forward, the specimen is female. Photo shows a male dolphin.

REGIONAL OBSERVER LIFE HISTORY REQUIREMENTS

	OFFSHORE SPOTTER	COASTAL SPOTTER	EASTERN AND WHITEBELLY SPINNER	COSTA RICAN SPINNER	STREAKERS	DELPHINUS	ALL OTHER
TOTAL LENGTH	X	X	X	X	X	X	X
COLOR PATTERN	5	5	5	5			
DORSAL FIN TRACE			X	X		X	X
PHOTOS					X	X	X
TEETH	X	X	X	X	X	X	X
HEAD		X		X	1	1	X
REPRODUCTION DATA	X	X	X	X	X	X	X
REPRODUCTIVE TRACT	2	X	2	X	X	X	X
STOMACH		X		X	3	3	X
LIVER		4		4			

X=COLLECT/RECORD

1-COLLECT HEADS OF ADULTS FROM BELOW EQUATOR OR OUTSIDE CYRA

2-COLLECT TESTIS/OVARIES AND UTERUS IF 150CM OR LONGER. DISCARD FETUSES NOT LEFT IN UTERUS (AFTER MEASURING AND SEXING)

3-COLLECT STOMACH FROM ALL AGES FROM BELOW EQUATOR OR OUTSIDE CYRA

4-COLLECT 200GMS AND FREEZE AS SOON AFTER DEATH AS POSSIBLE

5-RECORD COLOR PATTERN OF SPOTTERS, SKETCH COLOR PATTERN OF SPINNERS

Figure 8. Regional Observer Life History Requirements Chart

Table 1. Labeling, packaging, and storage requirements for some dolphin organ and tissue samples.

Sample	No. of labels Needed	Label Placement	Type Packaging	Type Storage
Testis or Reproductive Tract	1	organ	none	fixed wet (bucket)
Jaw sample with Teeth	1	jaw sample	none	fixed wet (bucket)
Head	3	a. mouth b. back of head c. outside bag	plastic bag inside burlap bag	frozen (wells)
Stomach	2	a. organ b. outside bag	plastic bag	frozen (freezer)

Lactating/Milk/Mammary Gland

1. Sampling Procedure (test for lactating)

The test to determine whether a female is lactating is in two steps. Locate the mammary slits on each side of the genital slit. Grasp each mammary, one at a time, between the thumb and forefinger and pinch firmly several times (Fig. 9A). If the pinch test fails to produce positive results, slice each mammary through the center to a depth of about two cm (Fig. 9B) and pinch again several times.

If no whitish fluid is exuded, enter "no" on the life history form in response to lactating. If fluid is produced by the test, inspect the incised area for cestode parasites. Cestodes sometimes encyst in the genital areas, encasing themselves in a membranous sac filled with a yellowish-white fluid that can be mistaken for milk. If you find no cestodes or other similar parasites and you suspect the fluid to be milk, enter "yes" for lactating. A blank means that you did not check for lactating, so do not leave this item blank unless you did not perform the test.

2. Utility

The presence of milk indicates either that the female is in the late stages of pregnancy or that she has been nursing a calf. If a non-pregnant lactating female is taken, the dependent calf probably would not have survived even if it had not been taken in the set. This information is important to biological researchers because it can be used in estimates of calf mortality.

Presently, there are no requirements for collecting milk and mammary-gland tissue. Procedural instructions will be issued if such sampling becomes necessary.

Teeth

1. Sampling Procedure

A sample from the middle of the left lower jaw containing between eight and ten teeth should be collected except when you collect the carcass in lieu of on-board processing. When the carcass is on its right side, the left mandible is fully exposed for examining and sampling. Open the mouth slightly and inspect the left tooth row. Locate the middle of the dental series. Make a 15-cm-long slice from below along the inside of the middle of the left mandible, so that the point of your knife protrudes into the mouth medial to the tooth row (Fig. 10A). Insert one of the blades of the pruning shears into the slit; then sever the jaw transversely four or five teeth in front of the middle tooth of the dental row (Fig. 10B). With another snip of the shears, sever the jaw behind the first truncation so that the jaw sample

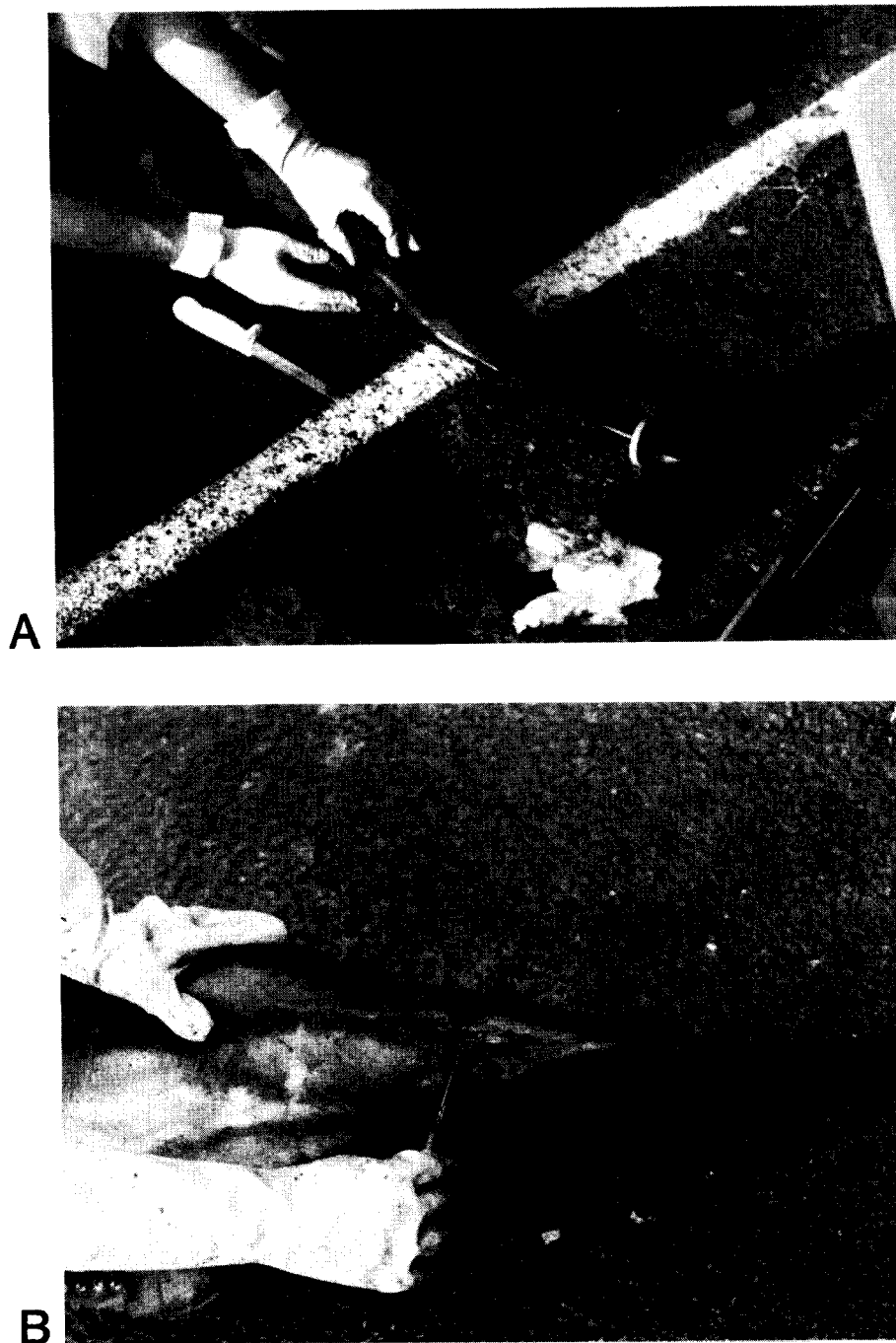


Figure 9. Two-step check of female dolphin for lactation. A. Each mammary is pinched several times. B. If no milk shows, each mammary is cut transversely to a depth of about two cm and pinched again.

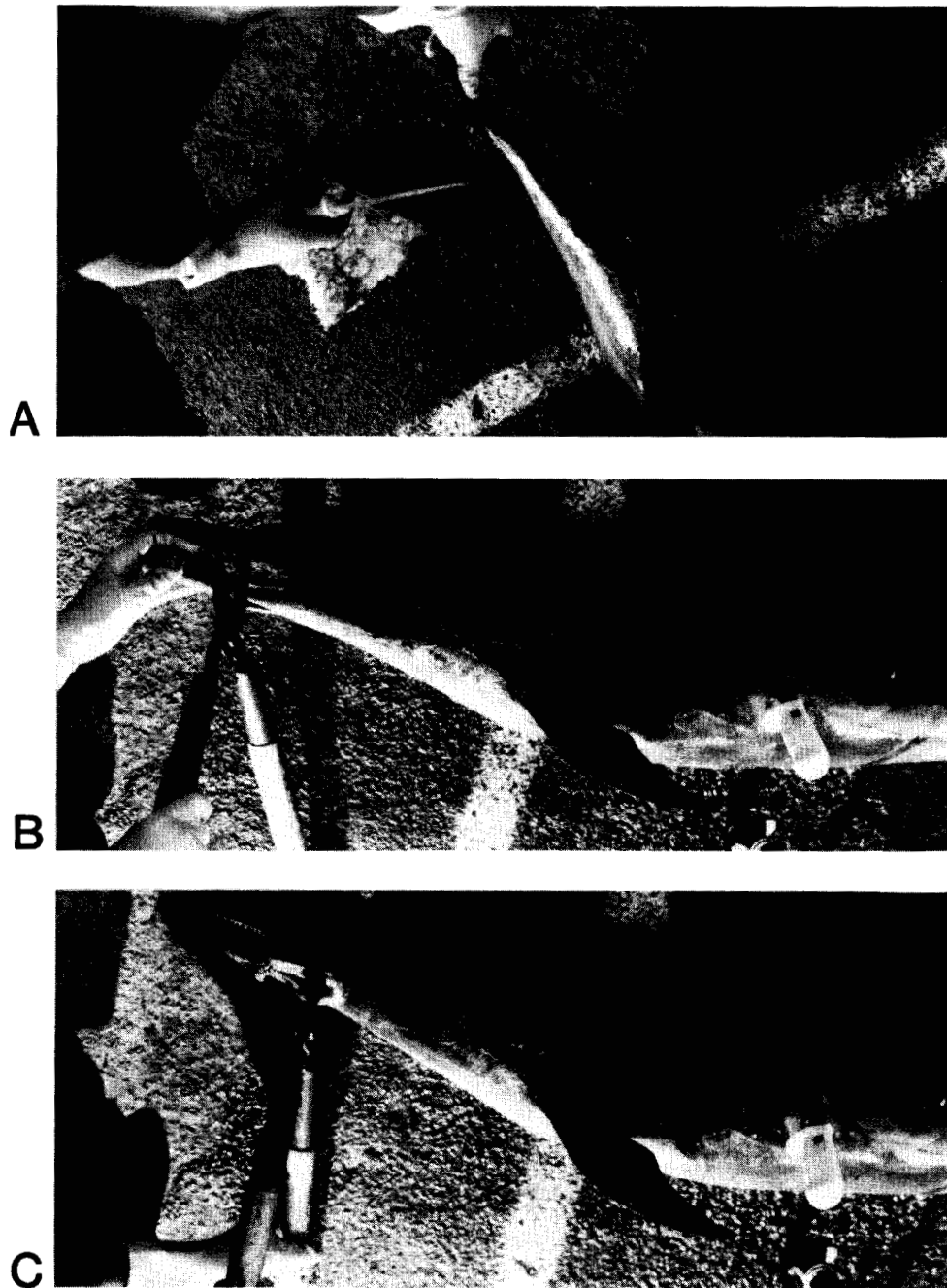


Figure 10. Collecting a jaw sample of teeth. A. A slit is made along the inside of the left jawbone. B. One blade of the shears is inserted into the slit and the jaw is severed transversely. C. The jaw is sheared again about eight to ten middle-teeth away from first truncation.

contains the required complement of undamaged teeth (Fig. 10C). The sample is now easily removed by using your knife to cut away remaining soft tissue connections. Label the jaw section once, record "yes" next to TEETH on the form, and place the sample into your sampling bucket.

2. Utility

After they reach the laboratory, the teeth are treated chemically, cut into thin sections, and read with a microscope to estimate the specimen's age. Ages from hundreds--even thousands--of specimens are analyzed together to try to determine whether the age structure or age-specific reproductive rates are changing within a given population.

The jaw sample is taken from the middle series of the mandible because that is where the clearest tooth layers form. The teeth are sampled from the left mandible for uniformity. Eight to ten teeth are needed because sometimes tooth preparations do not turn out well on initial tries and additional teeth must be used. A reserve supply is also desirable so that teeth may be available for future studies.

Head

1. Sampling Procedure

It is usually easiest to remove the head with the carcass resting on its right side if you follow the routine recommended. (First, collect and label a jaw sample.) Locate the blowhole on top of the head with your hand. Move your hand backwards to feel for the hard braincase beneath the skin and notice that the bony roof slopes downward at an abrupt angle posteriorly. The incision to separate the head from the vertebral column should be made just slightly (~3 cm) behind the sloped region. Make a small cut there with your knife blade. Make a small cut with your knife about 2.5 cm in front of the leading edge of the insertion of the left pectoral fin.

Sever the head from the body by connecting the two small cuts and deepening and lengthening this incision above and on both sides of the neck. As the incision progresses, pull the beak downward toward the specimen's belly (Fig. 11A). This expands the dorsal opening of the incision and allows you to feel for the juncture between the occipital condyles of the skull and the first (atlas) vertebra. Continue to cut as you pull the beak down and as the skull and the vertebral column separate, tension will be suddenly released (usually accompanied by a popping noise). Peek in, and if you have made a proper incision, you will be able to see the separation between the convex condyles and the concave articular surfaces of the atlas vertebra from the top of the incision (Fig. 11B).



Figure 11. Collecting the head of a dolphin. A. The initial incision is lengthened and deepened as the beak is pulled down toward the belly. B. The occipital condyles (arrow) pull away from the atlas vertebra; then the neck is girdled and the head completely severed.

Extend and deepen the incision to girdle the neck using short knife strokes. Continue cutting until the head is completely severed. Exercise care to ensure that no damage is done to the condyles.

The final task is labeling, packaging, and storing the sample (see page 16 for description). Enter "yes" next to the item HEAD as you complete the collection.

2. Utility

The head is collected because measurements of and relationships between cranial bones are among the most useful characters in diagnosing different races and subspecies. At the laboratory, the head is flensed, and the skull is cleaned and studied. Morphometric and meristic data gathered from the specimen are added to the appropriate data base for later stock discrimination and distribution studies.

INTERNAL ANATOMY ITEMS

Organization

The organ systems of delphinids correspond generally in position (but not necessarily in proportions or morphology) to those of most other placental mammals, i.e. eutherians (Fig. 12). The body cavity is partitioned transversely by a muscular **diaphragm** into a **thoracic** portion containing a four-chambered heart, trachea and paired lungs, the esophagus, and various major blood vessels including the dorsal aorta; and an **abdominal** portion containing a liver, a stomach, a spleen, pancreas, intestines, reproductive tract, and urinary bladder. Retroperitoneally, the abdominal cavity contains paired kidneys and paired adrenal glands.

Some of the features that distinguish delphinid internal anatomy from that of most other eutherians, however, include: 1) the numerous stomach compartments (non-ruminant eutherians typically have one), 2) the small number of lobes of the liver, 3) the lack of a gall bladder, 4) the peculiar grape-bunch-like (botryoidal) morphology of the paired kidneys, 5) the extensive system of blood vessels ("retia mirabilia") in the thoracic cavity and elsewhere, 6) the remarkably long intestines, and 7) in males, the internal testes. Despite these general differences, however, dolphin anatomy is very much mammalian and the identification and collection of internal anatomical samples from dolphins is easily learned, especially if you have had prior experience with mammalian or comparative vertebrate anatomy.

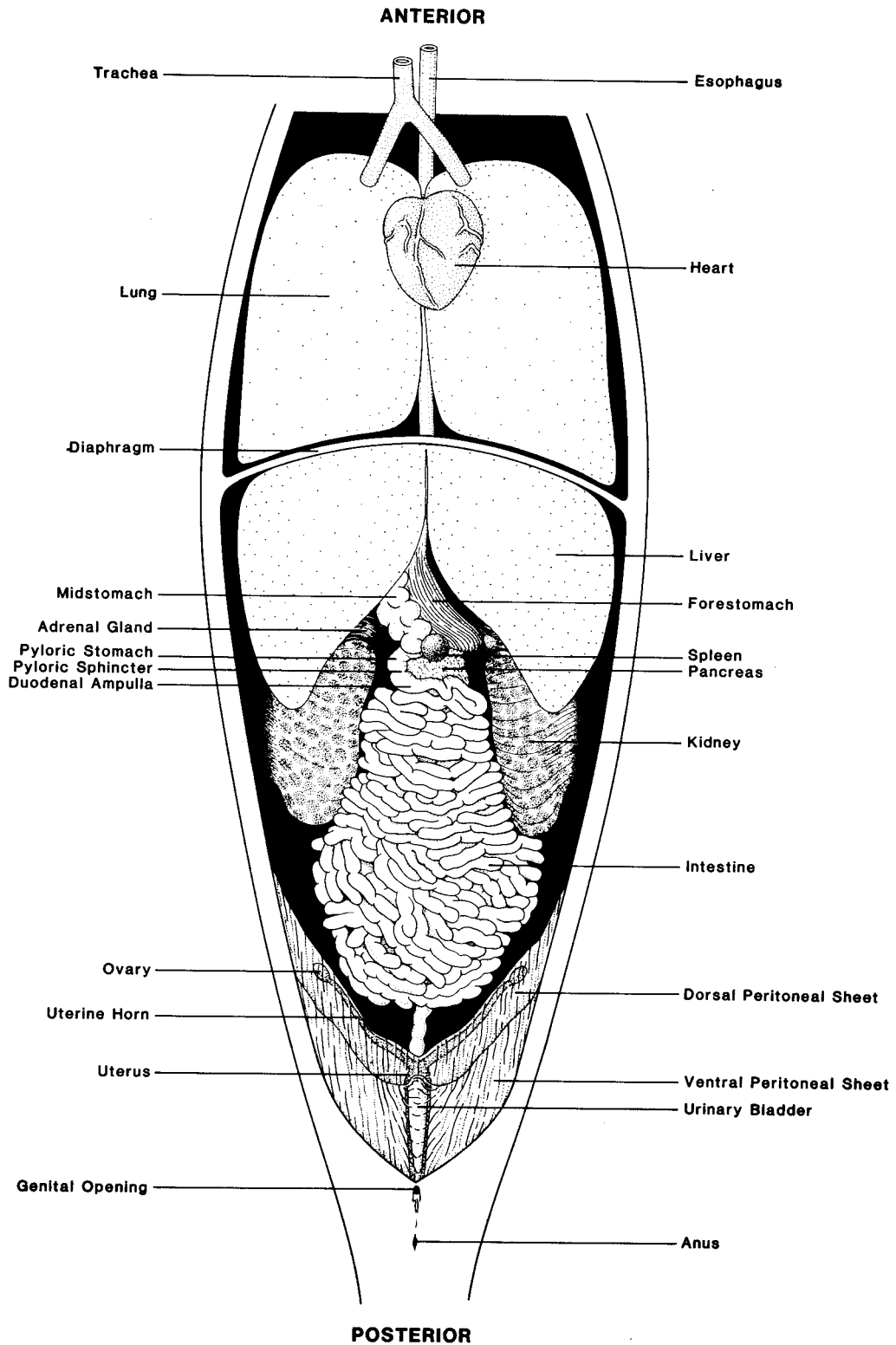


Figure 12. Line drawing showing organization of internal anatomy of a female dolphin in ventral view (length protracted).

Mid-ventral Incision

By now, all non-dolphin and external-anatomy items have been addressed and you have reached the point where you must collect the remaining data from inside the body cavities. For this, the carcass should be rolled high up on its right side, or as close to an upside-down position as possible. Observations and handling are best accomplished by straddling the specimen and bracing it with your legs. This allows both hands to be free for examining and cutting.

Inspect the venter for the **sternum** (the breastbone which the ribs are attached to in front) and the **umbilical scar** situated on the midline about halfway between the head and the flukes. The umbilical scar usually is visible as an oblong, discolored depression two to four cm in length, one to two cm in width, and a few mm deep (Fig. 6). Run your hand along the midline and you should be able to feel this hard knot of scar tissue surrounded by relatively soft skin and muscle. After having located the umbilical scar, you should note the position of the genital/anal slit(s) (Figs. 5, 6, and 7).

Short Incision

If your instructions require you to sample only the gonads, you may not have to cut as far forward as the umbilical scar. (Your own short-cut method for quick-sampling of gonads may be developed for such cases.) For the short version of the incision, straddle the carcass and face the tail flukes. Make a shallow, straight-line cut from a point about 15 cm behind the umbilical scar to the posterior margin of the anal slit (Fig. 13A). Once the length of the incision has been established, deepen it, but be careful not to damage the underlying organs that you intend to sample. Use the thumb or index finger of your free hand to hold the incision open (follow with your hand **behind** the knife) so that you can track the progress of the incision.

Your knife will first pass through the thin layer of skin, then the white layer of blubber, and then the red abdominal muscle (Fig. 13B). Finally, it will pass through the external lining of the abdominal cavity (**peritoneum**) and you should be able to see the abdominal organs covered by the thin transparent peritoneal tissue (internal lining), which can be cleared away with the fingers when necessary (Fig. 13C).

Extended Incision and Thoracic Flap

If samples of the stomach, blood, or other more anterior organs or tissues are needed, more working space inside the body cavity will be required than if you sample only the reproductive tracts. Both ends of the stomach must be found and tied off and you may have to locate and remove blood (probably) from the

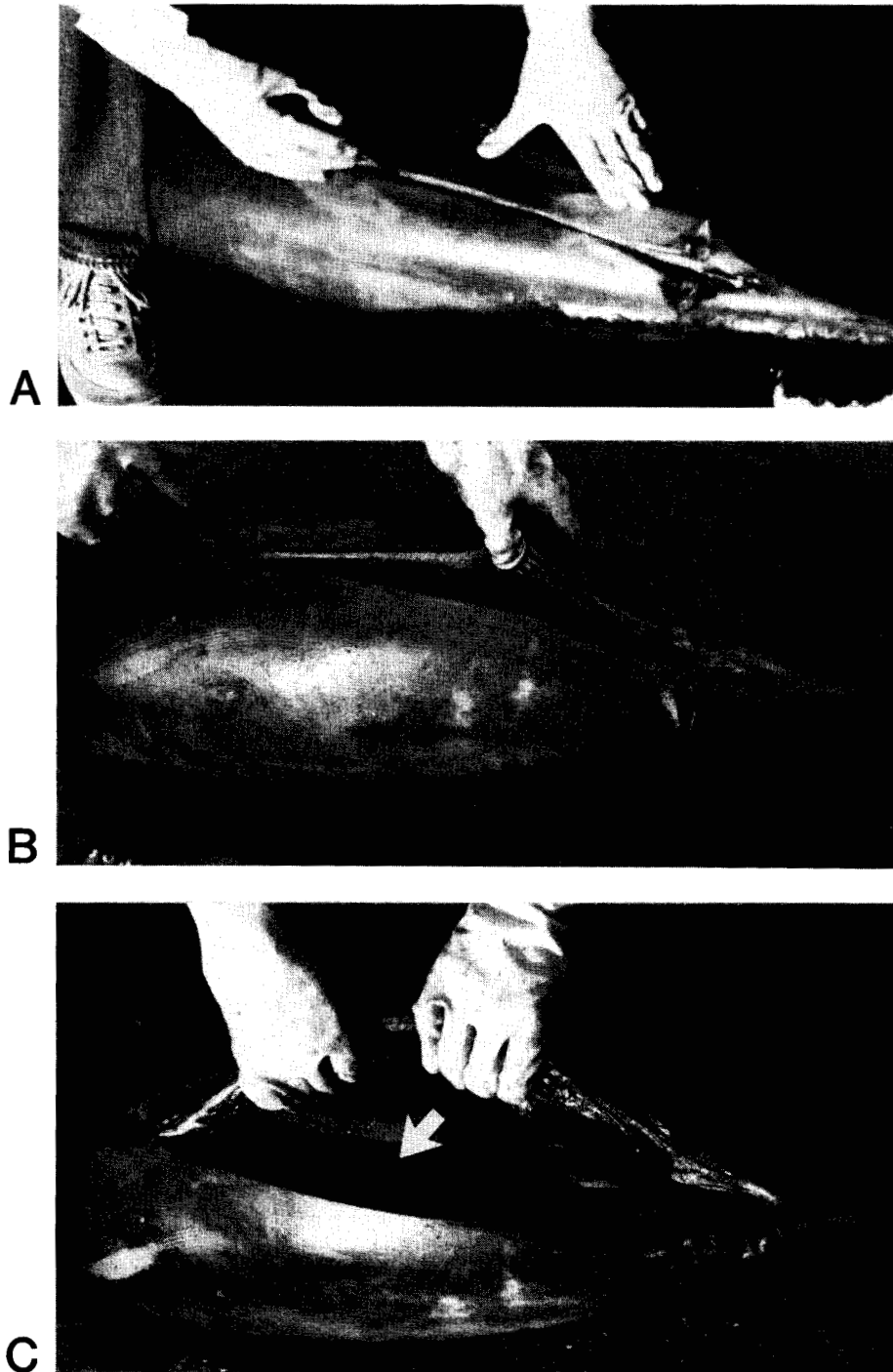


Figure 13. Short mid-ventral incision of female dolphin for collecting posterior abdominal organ samples. A. Incision extends from behind the umbilical scar to the anal slit. B. Incision is deepened. C. Incision completed. Internal abdominal organs can be seen (arrow).

dorsal aorta situated dorsal to the lungs. You will know immediately that the mid-ventral incision has to be extended anteriorly into the thoracic cavity. You will save time if you make the extended anterior cut at the same time that you make the initial incision.

Feel along the midline in front of the umbilical scar for the **xiphoid process** (posterior process) of the sternum. Make a straight-line cut from the posterior margin of the anal slit, through the umbilical scar, to the xiphoid process. Deepen the incision as in the short-incision procedure and stop (Fig. 14A).

Roll the carcass back onto its right side. After feeling for the junctures of the ribs with the sternum, extend the incision anteriorly from the xiphoid process by cutting forward through the cartilagenous rib attachments (at the base of the ribs) between the ribs and the left side of the sternum. To form a flap of ribs that can be reflected to expose organs in the thoracic cavity and the anterior portion of the abdominal cavity, cut upward on the left side between the ribs immediately behind the left scapula, i.e. shoulder blade (Fig. 14B).

Reflect the flap that you have just made and you are ready to collect samples from any part of the thoracic or abdominal cavities (Fig. 14C). As you raise the flap, notice the prominent transverse sheet of muscle, the diaphragm, that at once forms the floor of the thoracic cavity and the ceiling of the abdominal cavity. The diaphragm must be cut away from the left wall to fully raise the flap anteriorly, and one side of the sheet of peritoneum attached to the **urinary bladder** must be severed to raise the flap posteriorly.

Sampling from the Abdominal Cavity

Urinary Bladder

If the mid-ventral incision you have made is well-centered, the most ventrally positioned organ that you should see in the abdominal cavity is the urinary bladder. It is a heavily muscular, cigar-shaped organ, held in place (mid-ventrally and close to the body wall, immediately anterior to the genital opening) by a strong sheet of peritoneum that is attached to the wall of the body cavity on either side of the bladder (Figs. 12, 14C, 15A and B).

The bladder is a useful point of reference for you. If the carcass is female, the entire reproductive tract that you will be examining, labeling, and collecting will be located in a second sheet of peritoneal tissue immediately above (dorsal to) the sheet supporting the bladder (Figs. 12, 14C). During your initial workups, you may want to remove the bladder for a better view of the female reproductive tract, but there are no sampling requirements for bladders, so do not collect them. If the

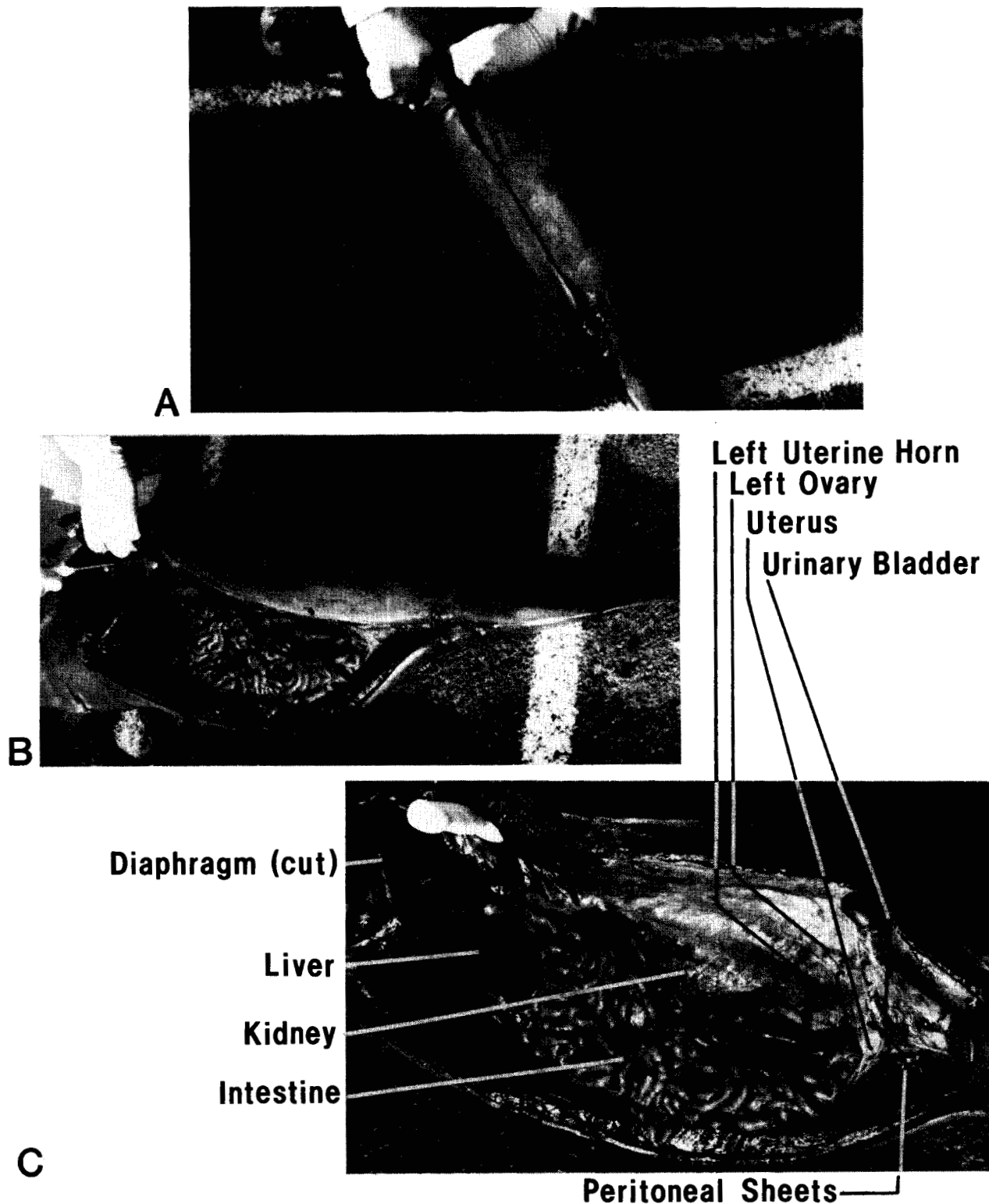


Figure 14. Extended mid-ventral incision for collection of organ samples from thoracic and abdominal cavities. A. Incision is made anteriorly from the anal slit to the sternum. B. Ribs are cut from their cartilaginous attachments to the sternum. C. The left side is reflected to expose organs of the thoracic cavities (white and gray knobs at upper margin of cavity are knife handles). Especially note the double sheet of peritoneum in the genital region. One sheet supports the urinary bladder; the other (more dorsal) supports the uterus.

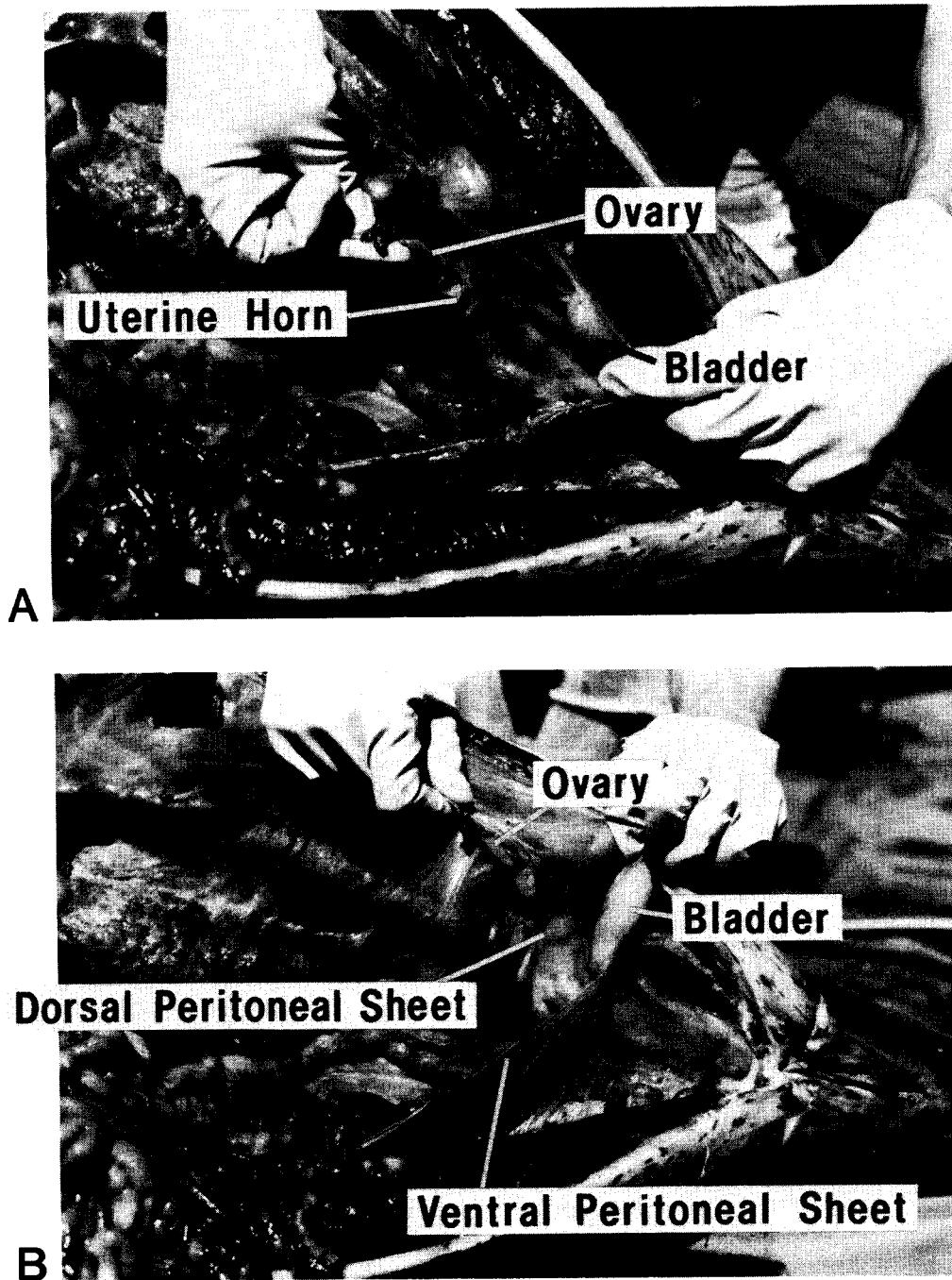


Figure 15. Position of the urinary bladder. A. Finger at right points to bladder, the most ventral abdominal organ. B. Bladder lifted to show sheet of supporting peritoneum.

specimen is male, you will be collecting only the right **testis** and **epididymis** located immediately dorsolateral to the bladder. Because the bladder's position does not usually interfere with the sampling of male reproductive organs, there is little need to remove the bladder in males.

Female Reproductive Tract

1. Description

You can best view the female reproductive tract and its sheet of peritoneal tissue (the **broad ligaments**) that holds the tract in place by excising the urinary bladder and its peritoneal support. The female reproductive tract includes the **uterus** (oriented along midline), a **right and left uterine horn** branched anterolaterally from the body of the uterus, and a small, bean-to egg-shaped, reddish-brown **ovary** delicately attached to the **fallopian tube** at the anterior end of each horn via the broad ligament (Fig. 12). The uterus opens posteriorly into the **vagina**, which is separated from the uterus by the **cervix**. The cervix (literally, "neck") is actually the posterior constriction of the uterus that, together with a number of circular folds in the lining of the vaginal canal, among other things, may function in sealing the uterus.

2. Sampling procedure

The standard procedure is to tag the left horn of the uterus and collect the entire reproductive tract. If carcasses are always sampled in the same position, you will always know which horn is the left one. Although the left ovary usually produces more ova and is larger than the right ovary in sexually mature females, exceptions are common and it may be impossible to distinguish left horn from right horn if one of the horns is not identified and labeled before removal. To avoid possible confusion, you should free and label the left horn before freeing the right one.

The physical connection between ovary and horn is tenuous and must be protected during sampling. While you cut, keep the left ovary and the anterior end of the left horn in your free hand. Pull them slightly away from the left wall. This makes the peritoneal tissue taut, enabling you to sever it more easily from the reproductive tissues. Draw the knife along the outside edge of the left ovary and horn and continue to cut the peritoneum away as you proceed down the left horn toward the uterus (Fig. 16A). Because the peritoneum covers the ventral surface of the uterus and uterine horns, it is easier to distinguish between peritoneum and horn if you intermittently evert the peritoneal sheet and examine its dorsal surface.

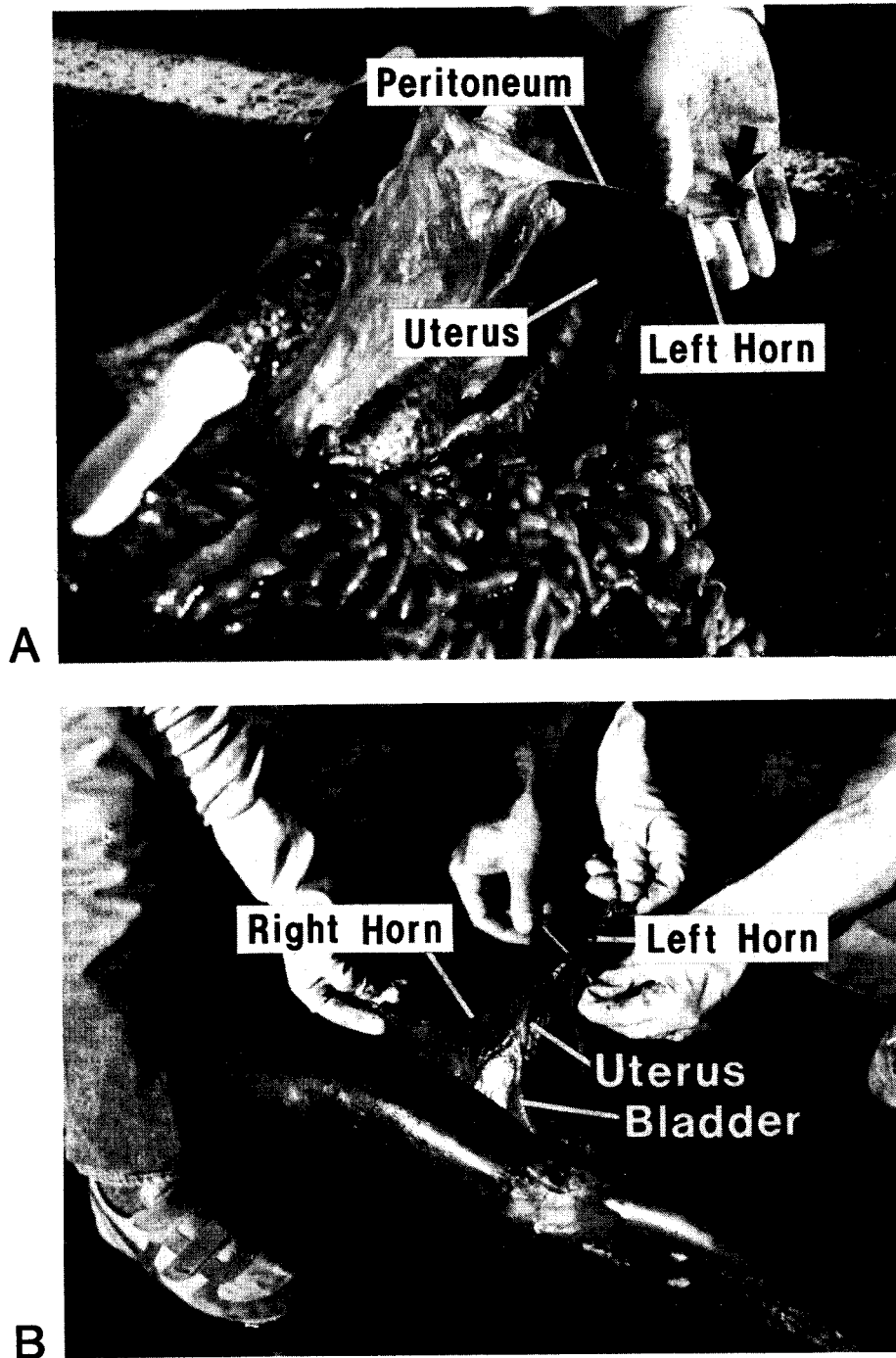


Figure 16. Freeing the left uterine horn and labeling it in place. A. The left ovary (arrow) and the end of the left horn are held for protection as the peritoneal tissue is cut away from them. B. Cutting is halted when the body of the uterus is reached. A cable-tied label is then cinched securely to the left horn, half-way between the ovary and the uterine body.

After the left horn is freed, attach a cable-tied label to it halfway between the ovary and the horn's juncture with the uterus and cinch it tightly to ensure that it does not slip on the horn (Fig. 16B). Free the right horn and all of the uterus, except for its connection with the vagina, in the same manner as you did the left.

In completing removal of the sample, use the cervical constriction as a natural seal to ensure that anything contained in the uterus is not lost. Locate the cervix by moving your finger up along the vaginal canal until you reach a firm, transverse, muscular barrier; then cut the reproductive tract off below it. Sever any connecting tissues and remove the sample. Now, place the reproductive tract on top of the carcass to be sure that all required parts are intact and that one horn (the left) is labeled.

3. Utility

Ovaries of dolphins contain data relating to their reproductive biology. Each time a female ovulates, a scar, "corpus albicans," is eventually formed on the ovary and it is generally thought that in dolphins corpora albicantia never disappear. A count of these scars provides an estimate of the number of opportunities a female may have had to become pregnant. Age estimates of females with only one scar, or only one freshly erupted Graafian follicle, furnish data about the average age that females of a certain population become sexually mature and how the age at sexual maturation may change. You are asked to tag the left horn so that technicians at the laboratory can tell left from right sides when they count ovulation scars on the ovaries and tabulate pregnancies for the right or left horn.

4. Pregnancies

Inspect the reproductive tract and feel for any firm mass inside the uterus or horns that may indicate a fetus. If a fetus less than 25 cm is found, leave it in the uterus and print "yes" for FETUS (print "no" if not; enter a question mark if you are not sure). Finally, tag the left horn, remove the reproductive tract, place it into your sampling bucket, and print "yes" beside UTERUS AND OVARIES.

It is usually obvious when a female is carrying a large fetus. Even before the ventral cut is completed, the fetus-filled uterus may begin to slide out of the body cavity through the incision (Fig. 17A). Without puncturing the uterus, make an approximate length measurement of the fetus through the wall of the uterus to see if it is less than 25 cm long. If its length is equal to or more than 25 cm, slit the uterine wall, remove the fetus, and sex and measure it (Fig. 17B). Before you discard it, record FETUS SEX and LENGTH data on the life history form and

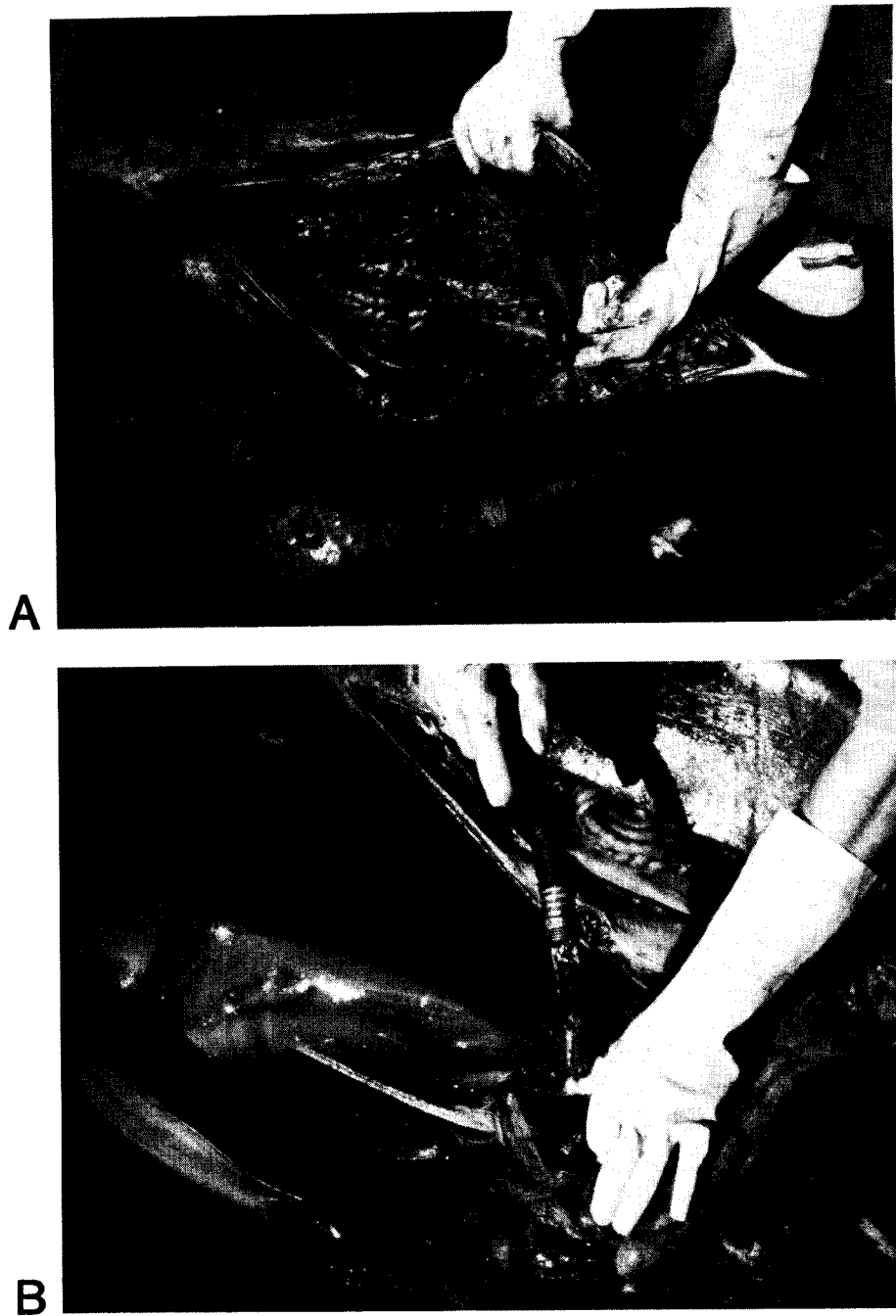


Figure 17. Handling large fetuses.

A. A nearly full-term whitebelly spinner dolphin fetus in the left uterine horn. The left ovary is in the observer's left hand. B. For fetuses larger than 25 cm, the uterus is slit and the fetus removed, sexed, measured, and discarded, but the uterus and ovaries are collected.

enter "no" beside FETUS [COLLECTED?]. Then, tag the left uterine horn, and collect the reproductive tract.

One of the most important pieces of life history data collected for assessing dolphin populations is the percentage of mature females that are pregnant in a mortality sample. Laboratory researchers estimate this by adding all of the "yes" responses in the FETUS [COLLECTED?] blank to all of the non-blank responses to FETUS:SEX and then dividing this sum by the total number of females in the sample that have at least one ovarian scar. Inferences about calving rates and trends in population growth for wild dolphins can be made from data on the percent of pregnant females sampled in the kill.

Male: Testis and Epididymis

1. Description

The testes are paired, sperm-producing organs, situated slightly posterior and lateral to the **kidneys**. They are surrounded by peritoneum from which they hang from the dorsolateral walls of the posterior part of the abdominal cavity, in somewhat the same position as the ovaries in the females. The dolphin testes are sausage shaped and range in length from a few centimeters in calves, to well over 15 centimeters in some mature adults.

The epididymis is a long, narrow, vermiform organ, with a sperm-storage function, that lies immediately dorsal to and in close association with the testis (Fig. 18A). The two organs are connected by a thin casing of peritoneum, which can be discerned by holding them up to a light and gently pulling them away from each other. The epididymis is longer on either end than the testis, and this sometimes creates a collecting problem with adult specimens because of the difficulty in defining the full extent of the epididymis posteriorly where it is continuous with the **vas deferens**.

The requirements for sampling male reproductive organs are to collect the **RIGHT TESTIS WITH EPIDIDYMIS ATTACHED**. The right side is sampled simply for the sake of uniformity. If you develop a sampling routine that includes placing all carcasses in the same position at the same stage of sampling, you will always know which organs are on the right side without having to stop and think about it.

If you move the intestines out of the way, along the back (dorsal) wall you should immediately see the paired kidneys that faintly resemble two bunches of grapes wrapped in transparent plastic. (Just anterodorsal to each kidney, is a small, firm, lobular **adrenal gland**--a potential candidate for future sampling (Fig. 18B).) If the carcass is that of an adult, the testes could be as large as the kidneys. The testes lie in almost the

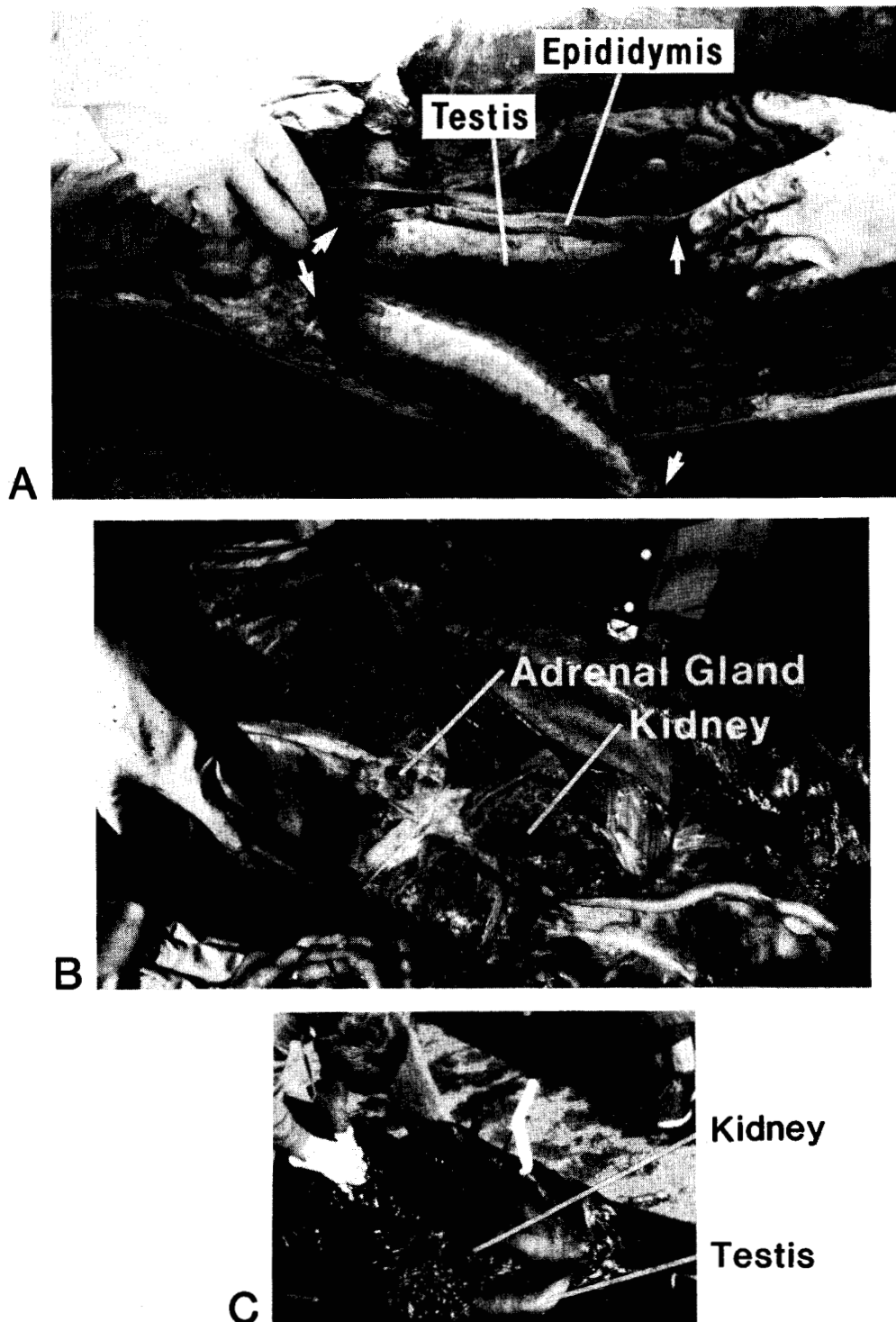


Figure 18. Paired organs of the abdominal cavity of male dolphins. A. Testes with epididymides. The epididymis is longer than the testis on either end (arrows). B. Kidneys and adrenal glands. C. Comparison of position, size, and shape of kidneys and testes of mature male dolphin. Testes are suspended from the lateral walls by peritoneum; kidneys are technically excluded from the body cavity by peritoneum.

same regions and are about the same shape as the kidneys, but testes have a smoothly textured surface as compared to the kidneys' lumpy or botryoidal surface (Fig. 18C).

2. Sampling Procedure

Pull the testis and epididymis away from the body wall with one hand. Beginning at the anterior end of the organ complex, use short knife strokes along the dorsal margin of the epididymis to free the two organs from their peritoneal connection with the body wall (Fig. 19A). When you reach the posterior end of the testis, you must determine the limits of the epididymis so that part of the posterior portion will not be cut off and left with the vas deferens. The epididymis and vas deferens are continuous organs and they are nearly indistinguishable from each other. Often, the transition between epididymis and vas deferens can be discerned by an abrupt thickening on the end of the otherwise tapering epididymis. This enlargement marks the anterior extent of the vas deferens and you may safely sever the epididymis from the vas deferens at this point. Whenever you are in doubt about the exact limits of the epididymis, collect part of the vas deferens too (Fig. 19B). Lab technicians can always discard the excess, but they can never regenerate what is missing.

Sever the testis and epididymis from all connecting tissue and remove them from the body cavity. Place the sample on top of the carcass and inspect it. If the testis is longer than six inches (~15 cm), it must be sliced to its center along its entire longitudinal extent so that the fixative will reach all parts of the organ (Fig. 19C). Large testes not prepared in this way will rot inside. Now, lift the sample by the middle of the epididymis and make a small slit with your knife just below, through the middle of the peritoneal connection between the epididymis and testis. Be careful not to puncture either organ. Thread a cable-tie, with a specimen-numbered label, through the slit and secure it tightly to the epididymis. Finally, place the sample in your sampling bucket and print "yes" next to the TESTIS item on the form.

3. Utility

In the laboratory, the testis is measured and weighed with and without the epididymis. Histological sections are made from each organ to determine the presence and abundance of spermatids and spermatozoa. The data are used in studies of male reproductive maturity and seasonality.

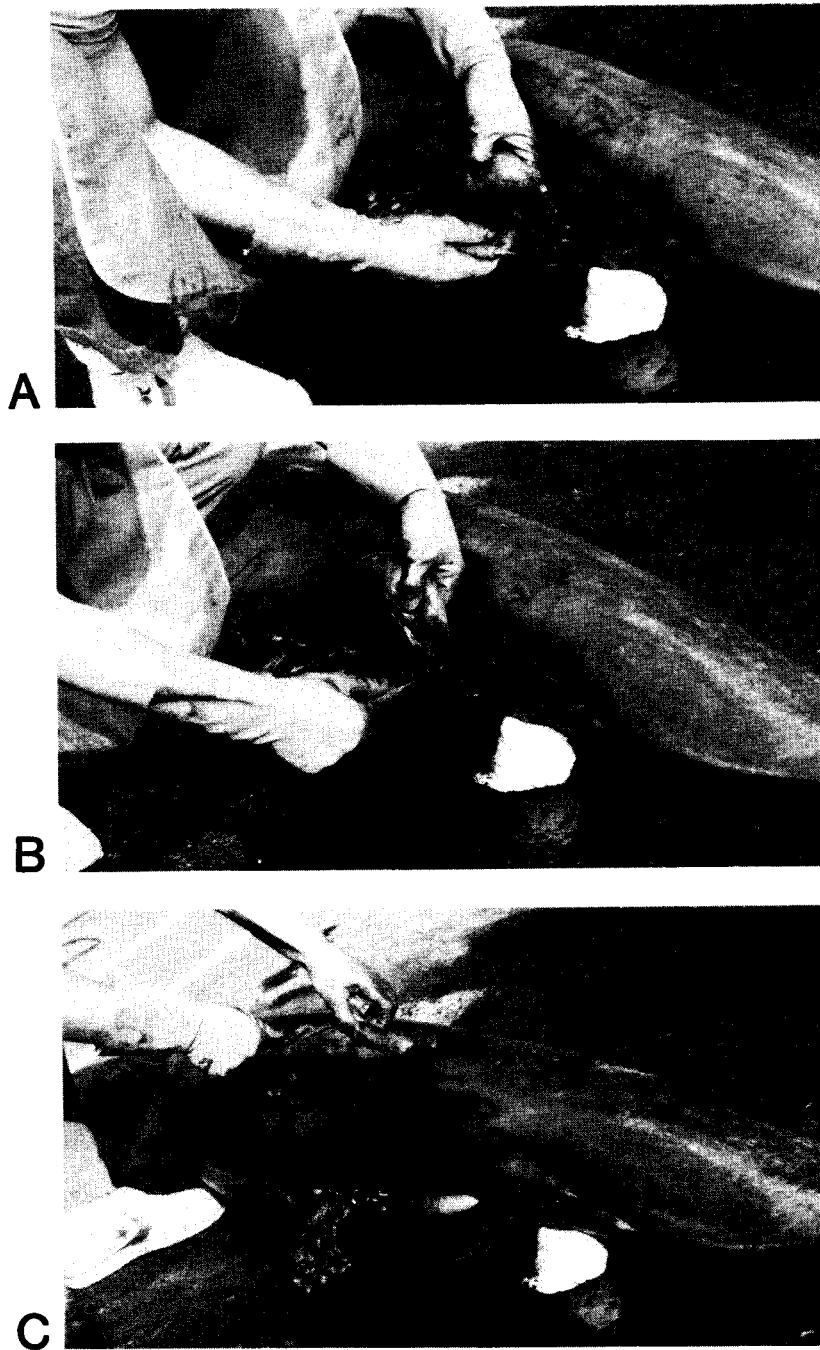


Figure 19. Collecting the right testis and epididymis.

A. The testis is pulled away from wall, making taut the suspensory peritoneum for easier cutting.

B. Peritoneum is cut along the outside of the epididymis and the posterior end of the organ complex is severed well behind its actual terminus.

C. Testes longer than 15 cm are slit lengthwise down to their centers to allow the fixative to penetrate the entire sample.

Stomach

1. Description

The stomach is located immediately under the diaphragm/liver complex in the anterior part of the abdominal cavity. As in other mammals, the delphinid stomach communicates with the **pharynx** anteriorly through the **esophagus**. The posterior part is in close contact with the **spleen** and **pancreas** and connects through the **pyloric sphincter** with the anterior end of the small intestine (i.e. the **duodenum**). Unlike that of other mammals, the delphinid stomach consists of three distinct compartments: 1) a highly muscular, non-secretory **forestomach**, 2) an enzyme-secreting mid- or **main stomach**, and 3) a thin-walled, non-muscular **pyloric stomach** (Fig. 12).

2. Sampling procedure

Because the stomach is collected for its contents, you should take every precaution to ensure that it is sealed and removed intact. Remember to store stomachs frozen because the stomach may contain calcareous fish otoliths and squid statocysts, important to prey studies, and formalin is acidic. If you have no instructions to collect the stomach, print "no" next to the item STOMACH on the life history form and go on to the next requirement. If your workup includes stomach collection, follow the steps below.

Give yourself enough room to work. Open the anterior part of the abdominal cavity sufficiently to follow the course of the digestive tract from the small intestine to the point at which the esophagus penetrates the diaphragm. If you have trouble locating the esophagus, open the posterior part of the thoracic cavity and cut through the diaphragm and liver to reach and expose the esophagus.

Define the limits of the stomach. Beginning at the esophagus, tease and cut away connecting tissues of liver, diaphragm, and peritoneum until you have cleared enough of the esophagus to close your free hand completely around the hind 10 cm of it (Fig. 20A). Carefully clear tissues from the three stomach compartments. The tissues and organs surrounding the pyloric stomach usually cause the most trouble. The walls of the pyloric stomach are very thin and flaccid and they are closely associated with the spleen (a compact, semi-spherical, darkly colored gland) and, especially, with the rather amorphous and flabby pancreas (Fig. 20B). The problem is further complicated by the system of twists and turns typically exhibited by the pyloric stomach and the duodenal (upper) portion of the small intestine. Pull gently on the pyloric stomach and clear away connecting tissue (mostly mesenteries, ducts, and blood vessels) with only your hands at this point. Follow the tract posteriorly until you reach the pyloric sphincter, a muscular constriction that

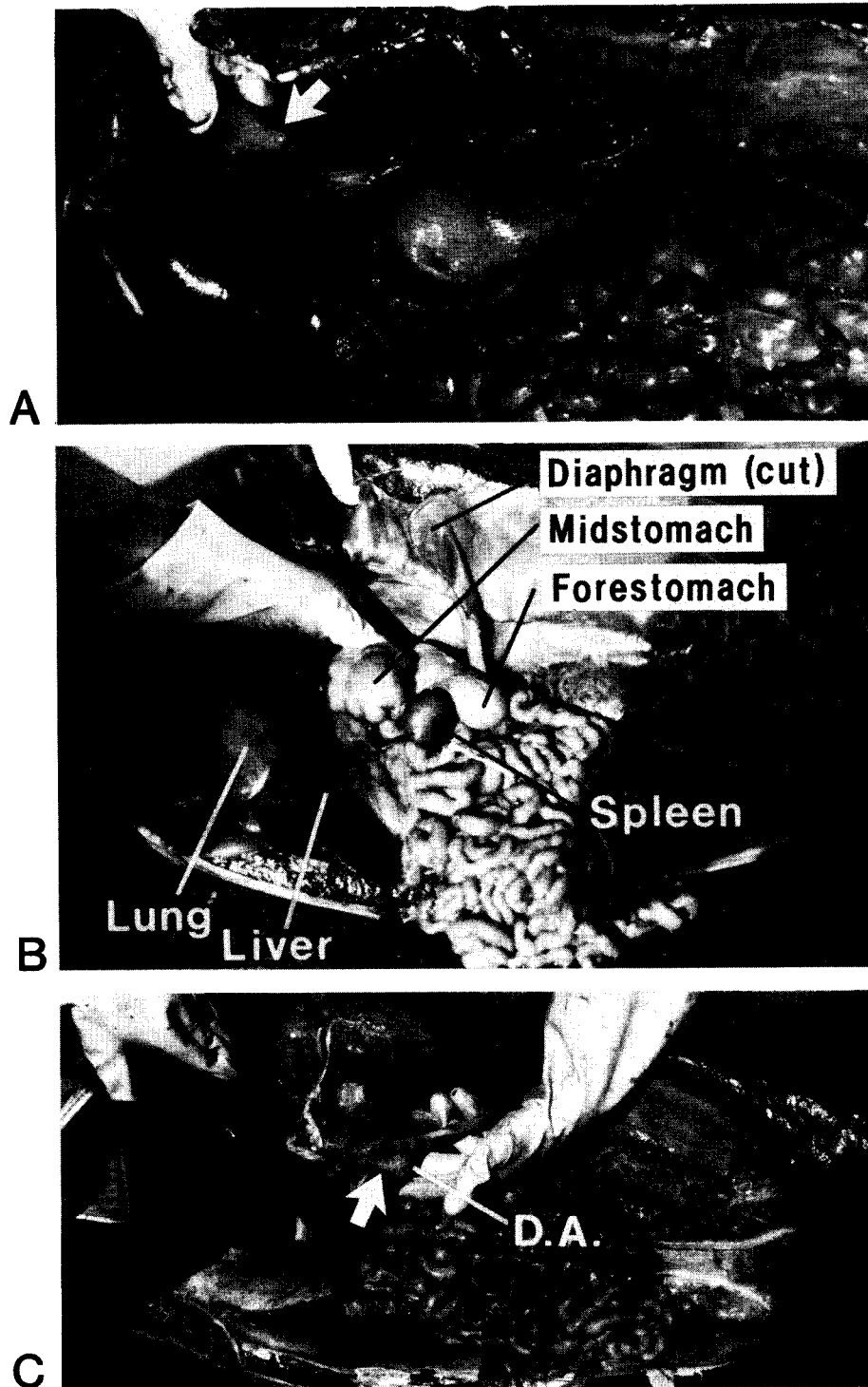


Figure 20. Defining the limits of the stomach.

A. The connecting tissues of liver, diaphragm, and peritoneum are cut away to fully expose the esophagus (arrow).

B. The tissues covering the three stomach compartments are removed.

C. The pyloric sphincter (arrow) and duodenal ampulla (D.A.) at the anterior end of the small intestine are found by searching along the posterior end of the stomach.

physically and functionally separates the stomach from the small intestine (Fig. 20C). The sphincter is identified by an abrupt change from flabbiness to firmness and wideness to a marked narrowness as you feel backwards along the pyloric stomach. Just posterior to the sphincter, the tract is wide for a short distance. This wide region of the small intestine is the duodenal ampulla. Posterior to the duodenal ampulla the intestine is (and remains) firm and narrow. Sometimes it is not easy to identify the sphincter or the duodenal ampulla. If you are unsure about the posterior limits of the stomach when it is time to remove it, tie off and sever the connection far down on the (more obvious) small intestine (Fig. 21A).

Close off both ends of the stomach. Because the esophagus and small intestine are difficult to seal off completely, it is important that you cinch down the cable ties as tightly as possible. Lock one around the esophagus about four or five cm above the stomach. Lock the other around the duodenum five cm or more below the pyloric sphincter.

You now are ready to begin the final cutting and clearing process to remove the sample. Sever the esophagus above the tie and hold the sample in one hand and pull it up (or roll it out) toward you as you tease away the connecting tissue (Fig. 21B). Cut off the spleen and discard it. You may risk puncturing the pyloric stomach if you try to remove much of the pancreas, so cut around the pancreas and collect it with the sample. Sever the connection with the small intestine below the tie and remove the sample from the body cavity (Fig. 21C). Finally, attach a cable-tied label to any part of the sample, place the sample in a small plastic bag, cable-tie a label tightly around the outside to close the bag, and put it with the samples destined for the freezer. After you have done so, print a "yes" beside the item STOMACH on the form.

3. Utility

Stomachs are collected in order to study what, when, and how much the animals ate. A stomach chamber may contain freshly captured prey, such as whole fish or squids, that can be readily identified and measured, or all chambers may be empty except for undigested hard parts from which prey size and species may be inferred. Data from analyses of large samples of stomachs can be used to address questions about resource use, species competition, prey-type and prey-size preference, and energy requirements of the sampled dolphins.

Major Organs of the Thoracic Cavity

The following discussion is included for general reference in case you are asked to collect samples from the thoracic cavity.

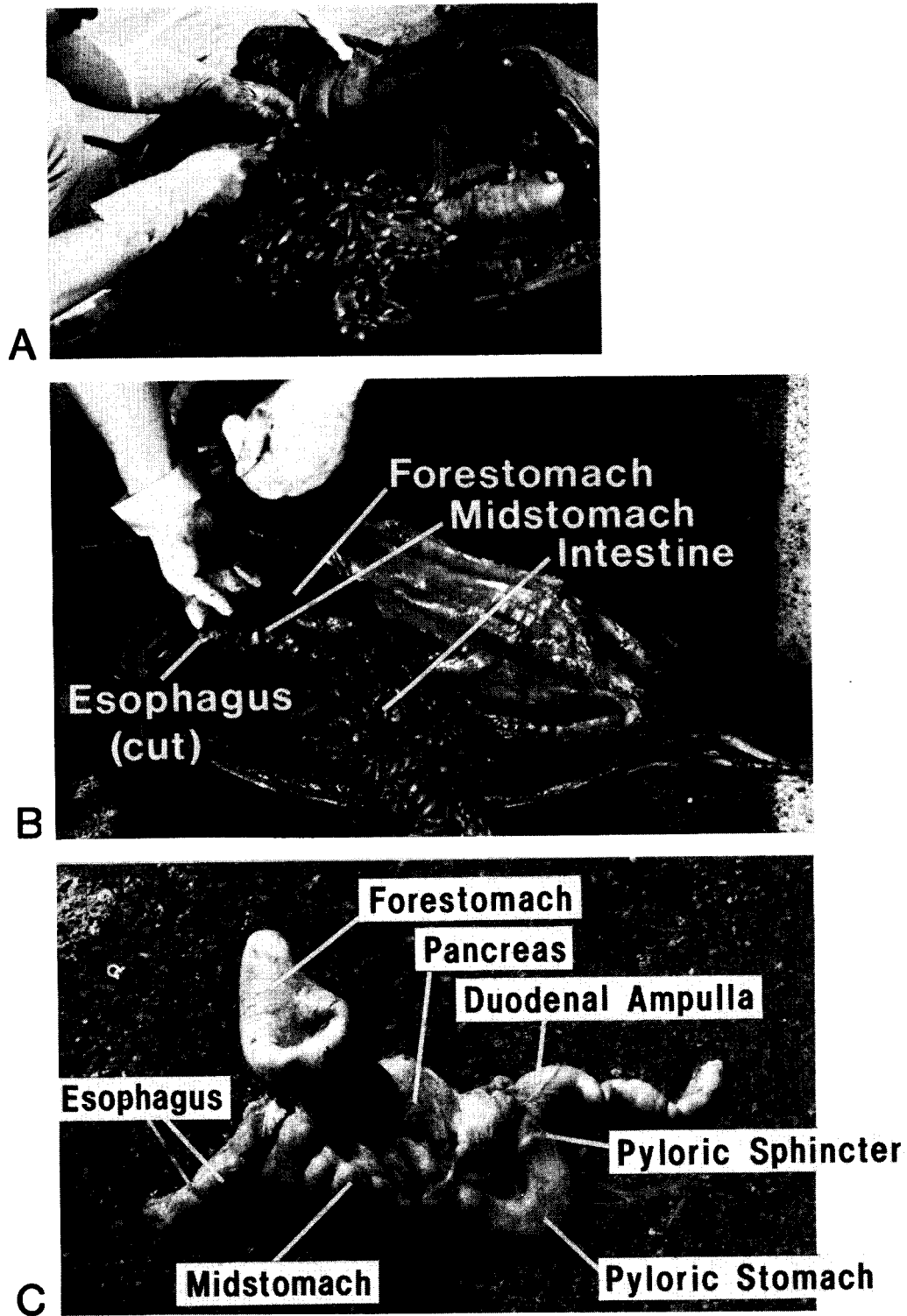


Figure 21. Removal of the stomach.

A and B. The stomach is tied off at the esophagus and at the small intestine below the duodenal ampulla. The esophagus is severed above its tie and the small intestine below its tie. The sample is lifted up and out of the cavity as the remaining connecting tissues are cut away from it.

C. The stomach is examined to ensure that it is intact.

Liver/Diaphragm/Lungs

The diaphragm forms the floor of the thoracic cavity, but it is in close association with the liver lying just inside of the abdominal cavity and with the posterior margins of the lungs that occupy the pleural portion of the thoracic cavity. When the diaphragm is cut back and cleared away, it is possible to mistake lung tissue for liver tissue, especially if you are in too great a hurry to follow the lobes medially to determine their origins. Inspect both tissues and note how closely they can resemble each other in gross shape and (sometimes) in color (Fig. 22A). Compare them for texture and resiliency by squeezing lung and liver lobes with your hand. Notice that the liver tissue is much more compact and muscle-like, while the tissue of the lung has more of a spongy consistency.

Heart/Dorsal Aorta

The heart lies ventromedially to (between) the lungs and is encased in the pericardium (or cardiac sac). Reach between the lungs and feel for this softball-sized, muscular organ. To expose the heart you must cut a hole in the tough sac and then open it up with your hands (Fig. 22B). Pull the heart out from between the lungs to inspect it. The largest of the four chambers of the heart is the left ventricle (Fig. 22B). If you are instructed to collect blood from the heart, you will likely take the sample from that chamber.

Aerated blood is pumped from the left ventricle to all parts of the body via the aortic arch. Blood is sent dorsally and posteriorly via the dorsal aorta which lies along the dorsal wall of the thoracic and abdominal cavities (Fig. 22C). Open the thoracic cavity wide with your hands and pull the lungs ventrally to expose this large artery. You may be asked to collect blood from the dorsal aorta if such samples are needed in the future. Precise details for blood sampling, handling, and preservation will accompany any blood collecting requests. Nevertheless, do not forget to answer item BLOOD with a "yes" or a "no" on the form.

Parasites

I recommend that you respond to this final "internal anatomy" item last (if it is still necessary), because parasites may be associated with any organ or area of the carcass and thus should be addressed when examining or sampling the applicable body part(s). There are no instructions for the sampling of parasites at the time of this writing, but you should remember to respond to this item with a "yes" or a "no" answer when processing a specimen. Special sampling requests will be accompanied by instructions when specific parasite samples are needed.

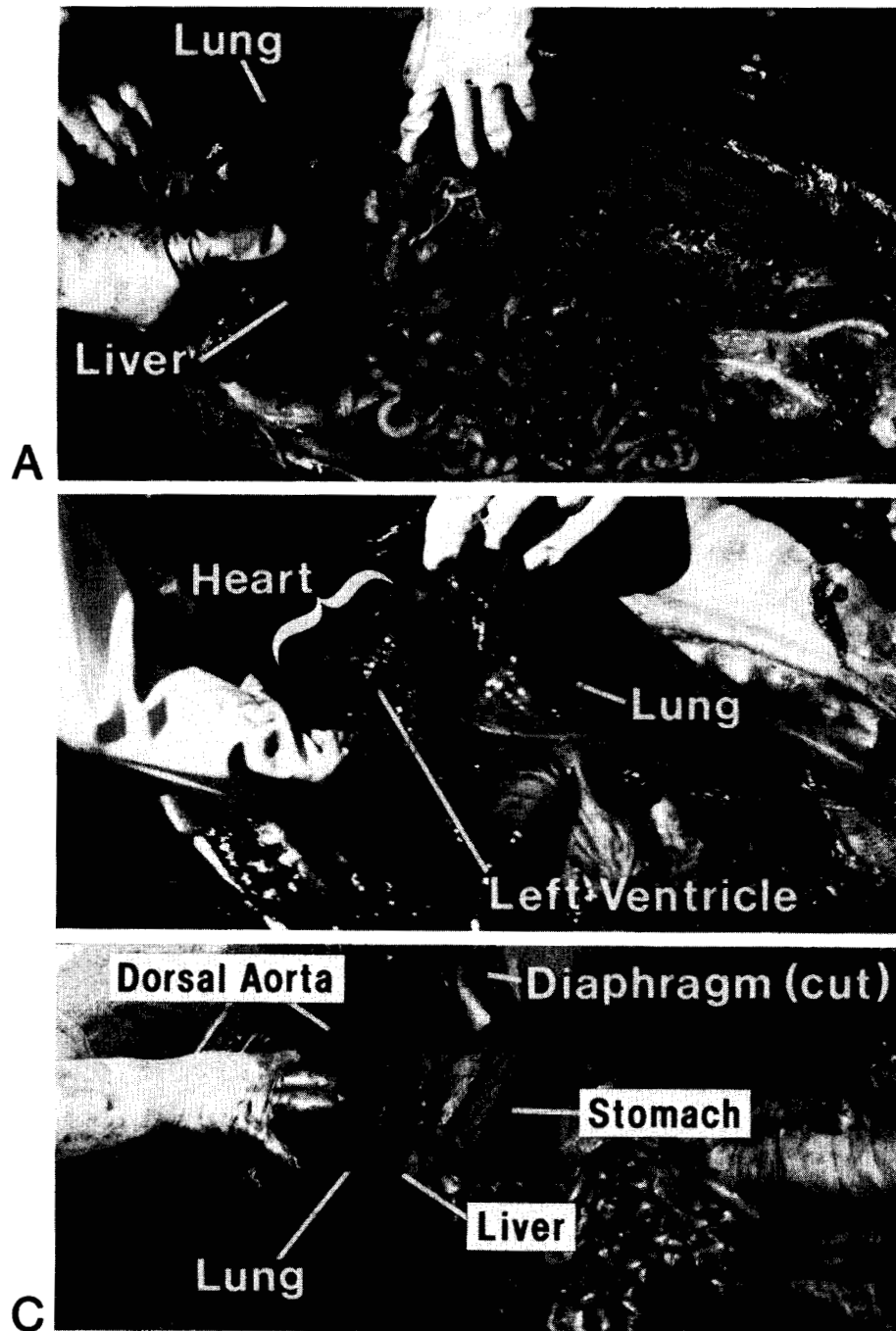


Figure 22. Major organs of the thoracic cavity.

A. The liver and lungs may be confused. They are similar in shape and size and sometimes in color. Lungs are spongy to the touch; liver is more compact.

B. The heart lies ventromedial to the lungs, encased in a sac of peritoneum. The largest chamber of the heart is the left ventricle. It is a potential source of aerated blood if blood is to be sampled.

C. The dorsal aorta lies along the dorsal wall of the thoracic cavity above the lungs and esophagus. This artery is a potential source for blood samples.

FINISHING

When you finish sampling the specimen, check your work. Make certain that you followed the requirements for the proper species, stock, and area. Check that you have responded to all of the appropriate data items on the form that you have just completed. Inventory your samples, and...remember to take the knife out of the knife holder before you discard it.

I hope that this handbook has helped to make your job a little easier and more relevant for you. You have a difficult job that not everyone can do. But it is an important job, indispensable to the research effort. Without the care and concern exercised by observers in making the collections, very few biological studies could proceed. You should take pride in that.

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